

Science has a word for it

"I'M SORRY, but arranging for you to see Dr Smith in the next few weeks is going to be very difficult; he's **delocalised** between London and Geneva". So said a scientist on the phone to us recently. The thought of the fairly ample Dr Smith being a mere probability density function and subjected to an uncertainty principle started a train of thought about how scientists could enrich or befoul the English language.

Some jargon has, of course, already made the jump, sorry the transition, from the laboratory to everyday use—for instance half-life. *Fall-out*, an undesirable consequence in its scientific context which became a desirable one in its general usage has got even further—to retirement. But there are riches untouched as yet, and others not well enough known. Here are a few examples.

world-line: 'If only I'd known you were in London yesterday, we could surely have made our world-lines intersect'. One way to have done this, of course, would have been to settle on a **time** and **space window**. That would have put up the **collision cross-section** by **orders of magnitude**.

hysteritic: Suitable as a description of management or bureaucracy. You push in one direction and you encounter resistance and delay; you reverse your direction of attack and lo and behold the self-same resistance and delay meets you.

boundary conditions: When you are looking around for a new job you specify certain requirements—rural environment, good schools in the neighbourhood, no overtime, easy access to London . . . In short, boundary conditions. A search through the classified advertisements then yields a set of suitable jobs consistent with them; presumably these are called **eigenfunctions**. The salaries, or **eigenvalues**, often bear a strong similarity to each other. This is hardly surprising given the nature of the world. But scientists even have a word for that; **degeneracy**.

boeing: Not strictly a scientific word, of course, but refers to the behaviour of many scientists. Many laboratories have one person who is always flying off to conferences. He is said to be boeing around. I bo, you

bo, he boes; though of course you and I don't do it.

orthogonal: A slightly derogatory term. 'His interests are pretty orthogonal to mine' indicates that of all the wealth of my interests, he can't find a thing in common with me. Other stronger insults are to imply that a colleague has **limited bandwidth** or, what comes to the same thing, **high-Q**—a narrow view of the world and an ability to go on and on when given the chance. Nor is calling someone **non-linear** polite. It suggests that under pressure they either fade or over-react.

deconvolution: Ask a simple question and some people, with complex **transfer functions**, convert your **input** into highly convoluted **output**. Deconvolution is simply finding out whether they said yes or no. You may also have a **signal-to-noise ratio** problem to contend with.

perturbation: invariably associated with an order. A **first-order** perturbation is very large indeed, such as getting married, having children, changing jobs. A **second-order** perturbation is trifling; for instance picking up a colleague on the way to work, answering the phone for him when he's out of the room.

tunnelling: Every graduate physicist knows that there isn't a potential barrier so high that it can't be passed through by hanging around outside it long enough. So to 'tunnel' is to get where you don't belong simply by persistence. Hence 'I see that at long last Jones has tunnelled his way into The Royal Society, the professorship etc. It sounds quite polite, even creditable, and suggests a certain amount of beavering; preferable to talking of Buggins' turn.

Do not confuse tunnelling with the ability of Hungarians to enter a revolving door behind you and come out ahead of you. This is a genuine and quite simple skill, and science has a word for it, too; **spin-flipping**. Those subjected to it have been known to remark 'What's happening to this spin-flipping door?'

Christmas competition. Now that we have started you on the learning-curve, we would like to access your database of similar examples. £10 for the most outrageous entry received at *Nature*, 4 Little Essex St, London WC2 by 6 Jan.





UK investigates virus insecticides

Pathogenic insect viruses may be a safe and effective alternative to chemical insecticides. The Unit of Invertebrate Virology at Oxford and the Centre for Overseas Pest Research in London are already conducting field trials with one class of virus: **Baculoviruses**. **A. J. McClelland and Peter Collins report**

THE EARTH is dominated by insects: they are present in staggering numbers (a billion billion, according to one estimate) and they are found in every habitable part of the globe. There is a greater diversity of insects than of any other group of animals, and they occupy many different, but essential, ecological niches. Fortunately, only a tiny minority, about 0.1% of species, are harmful to humans. This, though, represents about 3,000 insect species which can cause an immense amount of damage. Insect pests are vectors for several human diseases and also responsible for reducing available food supplies in the world by about 30%. Controlling them is thus of paramount importance in a world where food supplies are often unable to meet human needs.

For the past 40 years, chemical insecticides have been the primary method of controlling insect populations. This is because chemical insecticides were cheap to manufacture, easy to apply and highly effective. By comparison, biological methods of control appeared to be ineffective and unpredictable. But it is now realised that chemical insecticides are not the panacea they were once thought to be. First, the target insects have a remarkable ability to develop pesticide-resistance; second, chemical agents are not species specific and thus kill many other (often beneficial) insects in addition to the ones they were aimed at. Hence the increasing interest in the use of natural insect pathogens—parasites, bacteria, and viruses.

Of all the known insect pathogens, viruses appear to have the greatest potential for controlling insect pests. One group of viruses in particular—the Baculoviruses—seems to be especially

suited to this purpose since natural epizootics of these viruses provide ample evidence of their lethality for the larvae of their host species. However, attempts to use Baculoviruses to control insect populations have yielded mixed results. For example, the corn earworm (*Heliothis zea*) has been effectively controlled by viruses in the USA, but attempts to control the East African armyworm (*Spodoptera exempta*) have not been as successful. Those equivocal results were largely a consequence of the crude and haphazard methods that were employed. In some instances infected larvae were simply mixed with water, mashed up and used as a spray. Little research was done on the nature of the viruses, the dose required, or the possible side effects of the viruses on the environment.

The effective use of biological methods of control depends on a substantial knowledge of both the pathogen and the host. The Natural Environment Research Council's Unit of Invertebrate Virology (UIV) was established in Oxford under the direction of Dr T. W. Tinsley in order to obtain more knowledge about insect viruses. Tinsley is collaborating with Dr P. Haskell at the Centre for Overseas Pest Research (COPR) on a research programme to study Baculoviruses and conduct field trials to determine the efficacy of these viruses as insecticides. The aim is to establish the conditions under which the virological control of insect populations would be effective, reliable and safe, and thus provide an alternative to the existing methods of controlling insects with chemicals. Tinsley and Haskell's work reflects a widespread revival of interest in the concept of 'integrated control'.

Integrated control is the manipulation of the insect's ecosystem so as to reduce the population of a pest insect species with minimal disruption of the environment. The technique relies heavily on biological pathogens that are specific for the pest species, but does not preclude the limited use of other agents including certain non-persistent chemical pesticides. However, before viral pathogens can be used in such a programme, they must be fully characterised. To quote Dr Tinsley, "the indiscriminate release of replicating viruses into the environment is as reprehensible as the indiscriminate release of chemicals".

The UIV/COPR programme is divided into the following four stages:

- The development of methods for identifying, purifying and isolating viruses pathogenic to insects.
- Investigation of the possibility that a useful virus could infect species of insect other than the target species, or even vertebrates, particularly mammals.
- Laboratory testing of the efficacy of the purified viruses as insecticides.
- Field trials to confirm the results of laboratory testing and to determine the dose that must be applied to obtain high rates of kill.

According to Dr D. Knudson of the Yale School of Public Epidemiology, the UIV programme is unique in that virological research, research into the potential environmental effects of insect viruses, and field trials to determine the best way of using the viruses as insecticides are combined into a unified programme. The research at UIV is concerned primarily, but not solely, with Baculoviruses isolated from *Neodiprion sertifer* (the pine sawfly) and from four species of the genus *Spodoptera*, namely *S. exempta* (the East African armyworm), *S. littoralis* (the Mediterranean cotton worm), *S. frugiperda* (the US fall armyworm) and *S. exigua* (the US beet armyworm), which are major pests in the areas in which they occur.

●Left: Dying larvae of *Spodoptera littoralis* hanging from lucerne stems. Centre: Pheromone trap used during field trials to keep a check on the moth population and, thereby, the extent of virus infection. Right: Derrick McKinley of COPR inspects the trap.

Before any Baculovirus can be released into the environment, its ability to infect other species of insect and vertebrates, such as birds (which feed off the insects), as well as the ecological problems that may arise from the collapse of an insect population must be studied. Fully half the research effort at UIV over the last five years has been devoted to ensuring that Baculovirus insecticides are safe and will not harm the environment. To date no evidence of any potential harmful effects has been found. However, as a result of the research that has been done by the UIV, and others, quite a bit is now known about Baculoviruses, their host range, the way they infect their host and the effect they are likely to have on the environment.

It is known, for example, that Baculoviruses contain DNA and are large, rod shaped particles (about 100 nm by 300 nm). Experimental studies have shown that the majority of Baculoviruses are species-specific, although a few types have a slightly wider host range. However, all Baculoviruses are restricted to invertebrates and, as a group, they appear to have no chemical, physical or biological properties in common with any other class of virus.

The ability of the Baculoviruses to infect mammalian cells is being tested at Porton, the British laboratory that was originally established to do research into defence against 'germ warfare'. The results so far indicate that Baculoviruses do not infect mammalian cells and do not cause disease in animals. However, the details of the viral replication cycle are not yet fully understood and there is a possibility that Baculoviruses could induce the expression of a latent virus, for example an oncornavirus, in a mammalian species. That seems to be a remote possibility, in view of the restricted host range of Baculoviruses, and in view of the large number of experiments in which no covert Baculovirus infections in tissue culture grown mammalian cells could be detected, but it is now being investigated.

Immunological techniques for identifying and distinguishing among Baculoviruses from different insects have recently been developed by the UIV. This is an important development, since it enables the viruses to be monitored after they have been released into the environment. Thus, if the population of a species of insect, other than the target species, collapses after the re-

lease of a Baculovirus it can be quickly determined if the virus that was released is responsible or not. The goal of any insect control programme is, of course, to reduce severely or cause the collapse of an insect population. The environmental effects of inducing such a collapse with a Baculovirus insecticide can only be estimated by examining the effects of natural epizootics of these viruses.

Two spectacular examples of Baculovirus epizootics have occurred—one in Canada during the late 1930s and one in Wales in the mid-1970s. In both cases there appeared to be no measurable effects on, for example, the birds that normally fed on the target insects—they simply changed their diet. It is thought, therefore, that the environmental perturbations caused by species specific virus insecticides will be limited.

A substantial proportion of the research effort at UIV is concerned with the way in which Baculoviruses are disseminated under natural conditions, how they infect their host and how the natural infections are controlled. A characteristic feature of Baculoviruses that has been found to be important in their dissemination, and which also reduces their ability to infect vertebrates, is that they form polyhedral inclusion bodies which contain virus particles and which are highly resistant to disruption. The outer crystalline protein matrix of the inclusion bodies can only be disrupted at a very high pH (above 10) such as that which exists in the digestive tract of larvae. Insects appear to become infected with Baculoviruses by ingesting the virus and/or virus inclusion bodies with their food. However, the inclusion bodies cannot be disrupted by the human digestive tract (thus protecting humans from exposure to infectious virus) or by the digestive tract of birds. Inclusion bodies thus pass through the gut of birds that have fed on infected larvae and are disseminated in the environment.

The stability of the inclusion body has important implications for the way in which Baculovirus insecticides could be used. Since the infectivity of the virus inside the tough outer matrix is preserved it could mean that the virus could be readily stored as a dry powder or aqueous solution which need only be applied infrequently. On the other hand, it may be necessary to create an artificial epizootic every year. The only way to resolve this type of problem is to conduct extensive field trials.

Both the UIV and COPR have started field trials with Baculovirus insecticides. In Britain, the UIV has received clearance from the Ministry of Agriculture's Pesticide Safety Precaution Scheme to test the virus isolated from the pine sawfly (*N. sertifer*) to see

if it can be used to control this forest pest. In the COPR programme, preliminary trials using the *S. littoralis* virus have been done in Crete and a more extensive study of this virus will be started soon in the Egyptian Fayoum. The preliminary results of these trials are very encouraging. For example, it has been found that spraying viruses from a low flying helicopter is an effective method of controlling *N. sertifer*—probably because the down draught from the helicopter helps to distribute the virus.

In both the UIV and COPR trials novel ways of applying the viruses are being tested and, in keeping with the philosophy of integrated control, different combinations of agents are, or will be, used. An example of this is the proposed use of pheromones in Egypt by the COPR team. Pheromones will be used to attract male adult cotton worm moths (*S. littoralis*) into traps containing the virus insecticide. It is hoped that these moths will transmit the virus to the females during copulation who, in turn, will contaminate the outer shell of their eggs with the virus during oviposition. Since the first thing the larvae do after hatching is to eat their own eggshells, they will immediately become infected and die before they can do much damage to the crop.

The same rationale lies behind the concept of prophylactic spraying, enunciated by Dr Tinsley at the UIV. In this approach, low doses of viruses are sprayed before the eggs hatch. The advantages of this approach are first, that the larvae are killed soon after hatching and before they damage the plants or crops they normally feed on, and second, that since only low doses are needed to kill the hatching larvae, any possibility of upsetting the environment is minimised. The methods being tested in the field trials show clearly that the knowledge that has been obtained of the biology of the Baculoviruses and their insect hosts, is being put to good use.

Virus insecticides have the potential of being able to control many different species of insect. For the first time, therefore, we can envisage an alternative to the widespread use of chemical insecticides—and this at a time when chemical insecticides are becoming more and more expensive to manufacture.

In contrast, virus insecticides should be relatively cheap to produce, and should be within the capability of countries that do not have large petrochemical industries. Since a large proportion of the agricultural crops grown in the Third World are destroyed by insect pests, this research is potentially of enormous benefit to underdeveloped countries. □

US has doubts on anti-proliferation proposals

PROPOSALS presented jointly by the UK Atomic Energy Authority (UKAEA) and the US Electric Power Institute (EPRI) for reducing the proliferation dangers associated with nuclear power plans have received a less-than-enthusiastic reception from a number of groups concerned with US nuclear policy.

The proposals were announced at a meeting in Washington last February by Dr Walter Marshall, deputy chairman of the UKAEA, and Dr Chauncey Starr, president of EPRI. They involve a new system for reprocessing spent reactor fuel known as CIVEX which, it is claimed, makes the diversion of nuclear fuel virtually impossible by ensuring that pure plutonium is not accessible at any point of the cycle.

The proposed system is now being looked at closely as part of the International Nuclear Fuel Cycle Evaluation (INFCE) study, set up last year largely at the suggestion of President Carter. It has already been studied by a number of organisations in Washington, and received a variety of responses.

In the report of a study group set up to assess the CIVEX concept, the industry-based Atomic Industry Forum said that it found the concept to be "technically feasible" and "worthy of further development" and that it represents a considerable advance in answering fears that plutonium could

be diverted from the fast-breeder cycle.

However the study group points out that there are a number of technical and economic questions that must be resolved before CIVEX's true potential can be realised. And it adds: "A breeder cycle embodying the CIVEX concept does not significantly increase diversion risk in comparison with no breeders at all. Other routes for obtaining fissile material exist independent of the breeder and dominate the diversion risk in either case".

Considerably stronger criticism of the CIVEX concept was made by one of the five members of the Nuclear Regulatory Commission (NRC), Mr Victor Gilinsky, speaking at a recent conference in London sponsored jointly by the AIF and the British Nuclear Forum.

"It is difficult to take CIVEX seriously as an answer to the manifest danger of dispersing plutonium in weapons usable form, a problem we will face, according to Judge Parker, ten years from now; there is no conceivable way this scheme can be implemented within the period Judge Parker has allowed us," he said.

Drawing on internal studies that have been conducted by NRC staff, Mr Gilinsky, who was previously director of applied science and technology with the Rand Corporation, said that CIVEX fuel remained radioactive long

enough to be self-protective for a "fleeting period of time" compared to spent fuel. Its radioactivity was so diminished a few years after leaving the reactor that it could not provide the protection for which it was designed, he said.

"Since most spent fuel reprocessed in this century will have cooled for longer than that, the CIVEX process cannot contribute to the solution of the problems we must worry about now," Dr Gilinsky said. The "abortive flurry" over CIVEX, underlined the importance of attacking the problem of plutonium return now.

Equally harsh words have come from public interest groups. An evaluation carried out by Jim Cubie of New Directions and Tom Cochran of the National Resources Defense Council comes to the conclusion that CIVEX is "much less promising than at first glance", and that rather than being proliferation proof, it at best increases proliferation resistance.

"Even though it is intended for use in the next mid-century, because CIVEX has been claimed to make reprocessing acceptable, it will be used as an excuse for undertaking new plutonium reprocessing ventures in the next few decades and thus spread nuclear weapons capabilities," the two authors say.

David Dickson

Libya bidding to join nuclear club, scientists warn

LIBYA is actively seeking nuclear weapons even though it signed the nuclear non-proliferation treaty three years ago, warns the Federation of American Scientists. The FAS is urging the Soviet Union to cancel an agreement to sell Libya a 400-megawatt nuclear power complex.

Jeremy Stone, director of the Washington-based federation, said that Ahmed al-Shahati, who is head of the foreign liaison office of the People's General Congress, told him openly that Libya is still trying to obtain an atomic bomb. "Shahati made no bones about it, saying they would seek all weapons with which to defend themselves," Mr Stone said of his conversation in Tripoli in October. "To be sure I understood, I asked again: were they seeking to maintain the right to get a bomb or actually trying to get the bomb itself? It was the latter."

The Soviet Union, whose policy is only to sell nuclear technology to countries that have ratified the non-proliferation treaty, announced the Libyan contract in October. The Russians are currently negotiating safeguards with the International Atomic

Energy Agency to prevent nuclear fuel being diverted from the Libyan reactor to make weapons. Professor George Rathjens of the Massachusetts Institute of Technology, who is chairman of the FAS, estimated that the Libyan plant would produce enough fissionable material to produce "a couple of dozen" bombs a year.

In a letter to Anatoliy Dobrynin, the Soviet ambassador in Washington, Mr Stone asked: "Can the Soviet government rely upon the Libyan government to comply with the terms of the future

IAEA safeguards agreement if Libya cannot be relied upon to comply with the treaty itself?"

Libya's nuclear ambitions were well known in the early 1970s, when its leader Muammar al-Qaddafi reportedly tried to buy nuclear weapons from China and later said he wanted to purchase the bomb from anyone who would sell it to him. Mr Stone said these attitudes did not change when Libya cynically ratified the treaty to become eligible for the Soviet reactor.

The FAS has also written to President Carter, asking him to take the matter up with the Russians and raising the possibility of international sanctions against Libya. There is not much the US can do on its own, Mr Stone said, apart from sending home the 2,000 Libyan students in American colleges and universities (10% of whom are studying nuclear science.)

The federation also named four other "potential false adherents" to the non-proliferation treaty: Taiwan, South Korea, Iraq and Iran. But Mr Stone did not claim to have any direct evidence that any of them was blatantly seeking nuclear weapons like Libya. □



"I'm afraid, Colonel Qaddafi, you are suffering from a nuclear power complex..."

Czech chartists claim two died in nuclear accident

CHARTER-77, the Czechoslovak human rights movement, last week entered the nuclear debate, with an exposé of conditions at the Jaslovské Bohunice power station. According to Document 22 of the Charter movement (distributed abroad by the Palach press), employees at the power station have been compelled (under threat of loss of premium payments) to expose themselves to radiation levels considerably above the safety standards, while, in the course of the last three years, two serious accidents, one of them causing the death of two workers, have taken place at the station. Indeed, claim the Chartists, since the second accident in February, 1977, the station is still "temporarily" closed.

The Jaslovské Bohunice power station has a long and chequered history. It was constructed under the terms of the Soviet-Czechoslovak general agreement for nuclear energy cooperation of April 1955. In 1956, it was predicted that the station would be commissioned in "about 1962"—in fact, it went critical in 1972 and became fully operational the following year.

According to Dr Frantisek Janouch, who at that time was working at the Czechoslovak Institute of Nuclear Research, and who took part in the many professional and organisational discussions relating to the construction of the reactor, the project was essentially a Soviet proposal and was carried out under Soviet supervision. The station uses a 110 MW gas-cooled

heavy-water reactor. According to Dr Janouch, the Russians wanted to see if it was possible to construct an effective reactor of this type—leaving the details to be worked out by the Czechs and done at Czech cost.

In 1969, before the Jaslovské Bohunice station, known as the A-1, was completed, the Czechoslovak nuclear energy industry was switched, following a Soviet "recommendation", to light-water pressure reactors of the VVER type, for which the Soviet Union would supply "a substantial part of the main equipment". Whether this change of plan affected the final stages of work on the A-1 is not clear; it appears, however, from the Chartists' report that the projected automatic system for mounting new fuel elements was never brought into operation, and the mounting was done manually. Workers on the reactor were, says the report, "under psychological stress", often working a 16-hour shift instead of the six hours or less customary in "developed" countries. On 5 January, 1976, an error occurred in the mounting process. The element shot out of the reactor, under a pressure of 60 atmospheres together with a large quantity of radioactive CO₂. Since the emergency gas-traps and filters were insufficient for an accident of this magnitude, radioactive gas escaped into the atmosphere. In the area of the accident, emergency evacuation plans went into operation; unfortunately, one escape door had been locked, apparently to reduce petty thefts, and

two workers were suffocated.

Some six weeks later, however, disaster struck again (according to the Chartists). During the mounting of new fuel cells, the primary circuit overheated, the air-tight seal of the steam generator ruptured, and, as a result, the primary circuit, part of the secondary circuit and the working area all became contaminated. Radioactive material entered the drainage system of the plant and a stream in the vicinity has since had to be "fenced off" as contaminated.

During the repair work to the reactor, says the Chartists' document, safety levels of radiation were increasingly ignored, in an attempt to expedite the work.

The anonymous authors of the document urge nothing less than an open discussion and local referenda as to whether nuclear power stations should be constructed at all. This is all the more remarkable since uranium is Czechoslovakia's sole native energy source of any magnitude, and current plans envisage a nuclear expansion of 10,280 MW over the next 15 years, so that by 1990 over 30% of the installed generating capacity will be nuclear.

With such a major commitment to nuclear energy, the reaction of the Czechoslovak authorities to the report is predictably to deny everything. No such accidents occurred, they say, and even if they had occurred, they were under no obligation to make any public announcement.

Vera Rich

Canadian scientists confused by government moves on R & D funding

LAST week the Canadian Government appointed a new Minister of State for Science and Technology, Mr Alastair Gillespie. A brief occupant of the post when it was first established in 1971, Mr Gillespie will take over from Mr Judd Buchanan, who was promoted in a Cabinet shuffle to president of the Treasury Board.

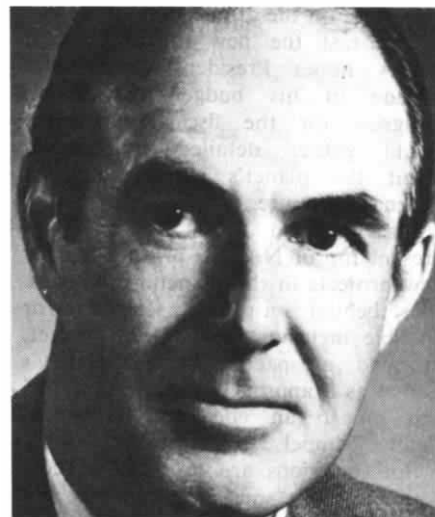
One of the new Minister's first tasks will be to sort out the confusion created in the minds of many Canadian scientists by recent moves over the funding of research. In particular, a strategy announced earlier this year by Mr Buchanan pledging greater support for research and development has been followed by budget cuts severely reducing various areas of federally-funded research, particularly in government laboratories.

R&D policy has recently become a hot political issue in Canada, figuring prominently in the pre-election cam-

paigns of all the main political parties. This prominence is based largely on the feeling that many of the country's economic problems have been due to its relatively low expenditure on research compared to other industrialised nations.

In June of this year, Mr Buchanan announced a number of funding increases and other measures designed ultimately, he claimed, to raise the proportion of the gross national product spent on R&D from 0.9% to 1.5% by 1983 (the comparable figure for countries such as West Germany and the US is about 2.5%).

In line with this strategy, a pre-election budget presented to the Canadian Parliament last month by Prime Minister Pierre Trudeau included a number of tax incentives to encourage private companies to invest in R&D. In particular, firms taxable at the small business rate will receive



Alastair W. Gillespie

a new investment tax credit of 25% on all R&D expenditure; for other firms, the basic rate of the R&D investment tax credit will be doubled from 5 to 10%.

The Government's declared commitment to increase R&D support have

been welcomed by Canada's Science Council, which has recently been arguing that such moves are necessary to promote the country's technological autonomy. "We are delighted with the new tax incentives, particularly those for small businesses, which tend to be an important source of industrial innovation, and where Canadian ownership is concentrated", the council's executive director, Mr John Shepherd, said last week.

Members of the scientific community, however, while welcoming the Government's commitment, are concerned that too much emphasis may be placed on relatively short-term research objectives, with a corresponding neglect of more long-range, basic research. And widespread concern was expressed in September when it was announced that a number of areas of federally-sponsored research would be cut back as part of a package of austerity measures.

The effect of the cuts, which included closing down a number of internationally recognised government research stations, has been to reduce the 1979/1980 research budgets of the

Department of Agriculture by \$3.5 million, of Energy by \$11.7 million, and of Fisheries by \$11.1 million.

Budget increase of \$5 million for the Natural Sciences and Engineering Research Council and \$3 million for the Medical Research Council, announced by Mr Buchanan in June will remain relatively intact, with only \$500,000 being taken away from each. However a \$2 million reduction in the budget of the National Health Research and Development programme will mean the termination of several health care research programmes.

The Canadian Association of University Teachers and other university-based organisations have sent a telegram to Mr Trudeau warning him of the "disastrous" implications of the cuts for higher education, research and health care.

Public expressions of concern have not been without their effect. Last month the Government reversed its decision to close one of the oldest research stations in Canada, that attached to an experimental forest at Petawawa, Ontario; following considerable outcry, it has been agreed to

keep the research station open, and to amalgamate into it two research groups currently based in Ottawa, namely the Forest Fire Research Institute and the Forestry Management Research Institute.

Nor has a number of universities been slow to realise that the future survival of their research efforts may depend on the extent to which they can build up a working relationship with both government and industry. This week, a two-day conference bringing together leading representatives of the three constituencies to work out mechanisms for co-operation is being held in Saskatoon, an event which some observers feel would have gained little support 10 years ago.

The scientific community must do more than simply complain about reductions in budget expenditures, says Dr John Kucharczyk of the CFBS, which has organised the conference. "Instead we must continue to stress the importance of a vigorous scientific effort to reach Canada's overall economic objectives."

David Dickson

NASA plans a new Venus mission

As US scientists prepare to analyse the data from the four probes that began their descent through the atmosphere of Venus this week, the National Aeronautics and Space Administration is already working on plans for a follow-up mission to the planet.

The current Pioneer Venus mission is primarily concerned with sending back data on the atmosphere of Venus. In contrast the new mission, which NASA hopes President Carter will include in his budget request to Congress for the fiscal year 1980, would gather detailed information about the planet's surface and its internal structure.

The proposed Venus mission is at present top of NASA's priority list for new projects in the planetary sciences. Close behind is a project, scheduled for possible inclusion in the 1981 budget, to send a spacecraft past Halley's Comet as it approaches the sun in 1985 and on to an encounter with the Comet Tempel 2.

Both missions are expected to cost between \$300 and \$500 million, and are within the framework of the US Administration's policy for space science announced two months ago. In a "normal" year, NASA officials feel the new Venus mission would stand a high chance of being financed; but with the country in a cost-cutting mood, it remains to be seen whether NASA's request survives the budget

process.

The new mission is known as the Venus Orbiting Imaging Radar (VOIR), and would involve a spacecraft circling the planet for at least seven months, providing the first detailed global view of its surface.

In the current mission, Pioneer Venus 1 will orbit the planet, and send back radar pictures. Their resolution, however, will not be much better than that which can already be obtained from Earth. The Soviet Venera missions have already sent back pictures transmitted from the surface of the planet, but only from three fixed locations.

The new spacecraft would, if it receives approval, be launched in December 1984 to arrive at the planet in May 1985. Radar mapping of the surface will be carried out using the synthetic aperture radar (SAR) successfully tested on the ill-fated Seasat mission; this will provide near-global coverage of the planet in moderate resolution, and coverage of a pre-selected small percentage of the planet's surface in high resolution (down to about 50 metres).

In addition to the SAR, the VOIR orbiter will also carry an altimeter and a magnetometer. It is hoped that the spacecraft will disclose the presence or absence of continents, ocean basins, volcanoes and other geophysical phenomena, as well as the nature and

time sequence of any plate tectonic activity.

The proposed mission to the Comet Tempel 2, which circles the sun once every five years, follows the abandonment last year of more ambitious plans for a close-range study of Halley's Comet, a mission for which NASA reluctantly decided it would have been too expensive to develop an appropriate propulsion system.

Part of the problem is that Halley's Comet circles the sun in an opposite direction from the earth, and a close encounter would have involved elaborate manoeuvring. The new plan is for a spacecraft propelled by the ionisation of mercury vapour to be launched on a course that passes close to the comet, dropping a probe into its tail, and then continues to a position that would allow it to travel alongside Comet Tempel 2 three years later.

Other projects submitted by NASA to the White House for possible inclusion in the 1980 budget include a gamma-ray observatory designed to study, for example, the high energy nuclear and gravitational processes which occur in the vicinity of neutron stars and black holes; an advanced X-ray astrophysics facility which will provide high resolution data on the position and structure of celestial objects such as pulsars, binary-star systems, and quasars.

David Dickson

news in brief

● **Birmingham University to be prosecuted:** The UK Health and Safety Executive is to prosecute Birmingham University following the smallpox outbreak from the medical school last August which led to the death of Mrs Janet Parker, a medical photographer, and was followed by the suicide of Professor Henry Bedson, who was head of the laboratory. A date for the start of the hearing has yet to be fixed; a spokesman for the HSE believed it would be early next year.

The university is to be prosecuted for "alleged failure to ensure, so far as was reasonably practicable, the health of its employees in the east wing of the medical school," according to a statement by the HSE last Thursday.

In a statement, the HSE reaffirmed the need for all medical establishments to make sure that the necessary precautions were observed in dealing with all category A pathogens, especially smallpox. The safety guidelines are contained in the HSE publication "Control of laboratory use of pathogens dangerous to humans".

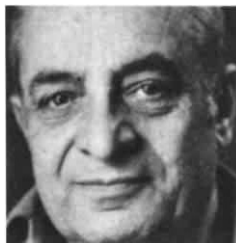
The HSE is to base its case on its own investigations. Meanwhile, Professor Reginald Shooter, chairman of the Dangerous Pathogens Advisory Group, is conducting an official government inquiry into the incident, which is also expected to be completed early next year.

● **US asked to reveal data on A-bomb tests:** The US Government has been asked to declassify all documents on atmospheric tests in the 1950s in an attempt to determine whether the tests were responsible for an abnormally high incidence of cancers in populations exposed to radioactive fall-out. The request has come from Governor Scott Matheson of Utah, who has expressed concern at an 11-year-old unpublished study by health officials which shows unexplained clusters of leukaemia victims in southern Utah in the path of fall-out from atomic bomb tests that took place between 1951 and 1962.

Thirty-eight claims have been filed against the Department of Energy by attorneys for survivors of cancer victims in southern Utah and Northern Arizona seeking more than \$1 million in damages for each of nine cancer deaths. It is expected that up to 250 further such claims will be filed in the near future. The claims are the first to have been filed against the government on behalf of civilians not directly connected with the bomb testing programme, but merely living in the path of radioactive clouds.

Last week a federal judge in Newark, New Jersey ruled that the US Army must surrender films and recordings of atomic bomb tests conducted in 1953 in order that a 47-year-old cancer victim, who says he was forced to witness a blast, can compile evidence in a \$13 million negligence suit.

● **£350,000 science awards:** Victor Hasselblad (right), inventor of the famous Hasselblad camera, who died earlier this year, left part of his fortune to establish awards similar to the Nobel prizes. From March next year, prizes worth a total of £350,000 are to be awarded each year to selected scientists for research into the natural sciences. The awards will be chosen by the Erna and Victor Hasselblad Foundation. According to a government official in Stockholm, Victor Hasselblad's instructions for the selection of scientists and the areas of the natural sciences for which the awards will be given, are in a sealed envelope which has yet to be opened by the executors of his will.



● **Lerner still cannot leave Russia:** Dr Aleksandr Lerner (left), the Moscow cyberneticist, has taken over the unenviable position as doyen of the *refusnik* scientists, now that Veniamin Levich has finally arrived in Vienna. Lerner, who is 66, has been waiting for an emigration visa for Israel for seven years. In the meantime, he has been working privately on a project for an artificial heart.

According to his daughter Sonia Levin, who visited London last week, Dr Lerner has now completed the basic control programme and the main priority is now for him to be able to collaborate freely with haematologists to establish the reaction of the blood to such an artificial organ. This, however, is not possible in the limbo conditions of a *refusnik* and the Soviet authorities have intimidated Dr Lerner that they would rather see him inactive in Moscow than doing useful work elsewhere.

Furthermore, some kind of personal reprisal seems involved due to Dr Lerner's activity in the Jewish emigration movement. US Congressman Rhodes, leader of the Republican party in the House of Representatives, says he was informed by the Soviet ambassador to the USA, that Dr Lerner would never be granted an emigration visa, because he is a "trouble maker".

● **Lead absorption higher than expected:** Scientists at the Atomic Energy Research Establishment at Harwell have shown that the proportion of lead from car exhausts absorbed by the human body is higher than previously expected. Denis Howell, Minister of State for the Environment, said: "This is an important finding and may be particularly significant where people are exposed to high levels of airborne lead from petrol."

The scientists confirmed that the majority of lead in blood comes from eating and drinking; lead from the air generally contributes less than 10% of the body's total uptake. However, at high concentrations, for example, people living near the busiest stretch of the M4, the contribution of air lead is comparable to that from diet. Sixty per cent of the lead inhaled by volunteers sitting near a motorway was deposited in the lungs. This finding agreed with earlier estimates. But the percentage of lead deposited in the lungs for people near urban roads was much higher than expected, at 48%.

The effect of lead on health, which was not considered by the scientists at Harwell, is to be assessed by a working party of independent experts which has been set up by the Social Service Secretary.

● **Kennedy plans hearings on biomedical research:** Senator Edward Kennedy has announced that the Senate health and science subcommittee of which he is chairman will hold a series of oversight hearings into the funding of biomedical and behavioural research early in the next session of Congress. Speaking during a ceremony to present the Albert Lasker awards for public health service and basic medical care research, Mr Kennedy said that in 1977 the US invested only 3.4% of its "health care dollar" in biomedical and behavioural research, compared with 4.9% in 1967.

"That decline in our overall commitment to research is short sighted and regrettable. It cheats our health care system of potential breakthroughs which will ultimately permit us to restrain health care costs without harsh regulatory controls. It has become apparent that our government has no clear system for setting priorities."

Risk assessment is more than numbers, Swedish seminar concludes

By Wendy Barnaby

"NEAR Santa Barbara, California, plans have been made to construct a large liquid natural gas terminal . . . A small group of American Indians is protesting the siting of the plant, contending that the chosen location is the most sacred spot of their culture, the Western Gate, where the Indians say the souls of their people must pass after they die to join the spirits of their ancestors . . . One can envision a Rasmussen-like report, examining the probability that a soul would be unable to migrate past a liquid gas terminal. The report would, no doubt, conclude that the risk was very small, perhaps 10^{-9} for each soul. To technical experts the risk seems negligible; to the Indians the risk is unthinkable . . ."

How can risk impact assessment (RIA) deal with value judgements? This emerged as one of the main themes of a three-day long seminar entitled "Impacts and Risks of Energy Strategies: Their Analysis and Role in Management", held recently in Stockholm. The answer, simply stated, is that nobody knows.

Scientists evaluate the risks involved in a proposed course of action; policy-makers draw up policies after they have weighed the risks in a social context; organisations carry out the policies—so the theory goes. The trouble is that this cycle involves scientific, social, psychological, political and managerial processes and disputes, and it is very hard to say how—or even by whom—they can all be brought together in some coherent whole.

According to Dr Baruch Fischhoff from Decision Research in Oregon, USA, RIA is limited by not having some inputs necessary for the analysis, by the analysts' inability to assess the validity of their work and incorporate that assessment into guidelines for action, and for its failure to address critical management issues. Some of the missing inputs are the preferences and values of the people who will be affected by whatever action is proposed: there is no adequate way of incorporating them into the numerical calculations that RIA demands. David Pearce, Professor of Political Economy from the University of Aberdeen, examined the issues that arose in the Windscale inquiry into whether or not to build an oxide fuel reprocessing plant in Cumbria, England, to see what—if anything—RIA could contribute. It is, he concluded, a valuable tool for ordering thoughts, but in considering issues like constraining the liberty of the

individual or risking nuclear weapons proliferation and war, it has little or no role to play. RIA is, he inferred, unable to deal with ethical and political choices.

The problem of who should evaluate what in the RIA cycle was nicely illustrated by a difference of opinion between Dr Timothy O'Riordan from the University of East Anglia, and Mr Carl Tham, now Sweden's Minister for Energy. Arguing that the technically-sophisticated identification and estimation phases of RIA have been more fully developed than the evaluative and control phases, Dr O'Riordan said, "Ideally, the basis upon which key decisions are made in the evaluative and control aspects . . . should be made fully public and should be subject both to extra-parliamentary and parliamentary debate." Taking the academic's view, he saw this as a means of incorporating values into the cycle, to counter our present blind faith in scientific advice. Mr Tham, on the other hand, recommended quite a different procedure. "Politicians or decision-makers are best served with hardware facts about risks and their impacts on health and environment", he said. "Then it's our (ie the politicians') job to evaluate these facts as sensibly as possible and to make the necessary appreciation of costs and benefits." But can the politicians assume that they understand how the public perceives issues? There is "an immense gap between the views of technical experts and the views of a significant portion of the public", concluded Drs Paul Slovic, Sarah Lichtenstein and Baruch Fischhoff of Decision Research after studying opponents of a nuclear reactor to see how they perceived the threats it posed. Are the politicians informed and impartial enough to make their policies reflect these perceptions?

The RIA cycle does not end with the policy-makers, either: management, generally in the form of organisations, then takes over. But there is no guarantee that what is actually done will be even vaguely like what the policy-makers intended to have done: a gap demonstrated by Dr Shelagh Staynes from London University's Monitoring and Assessment Research Centre, who talked about the administration of lead pollution control in the UK.

A more surprising difficulty with RIA is that there is so little communication between the different groups involved in it. The seminar—held jointly by the International Institute



Reprocessing at Windscale: the risk may be high or low, but how to deal with ethical and political choices?

for Energy and Human Ecology (the Beijer Institute) and the Swedish Energy Research and Development Commission in association with UNEP—did at least get these groups talking to each other. Pointing out that natural scientists, politicians, psychologists and ecologists all contributed to the meeting, Professor Gordon Goodman, the Institute's Director, said, "There have been few occasions—it happens almost never—when people quantifying objective risk have talked to people trying to measure a democratic society's evaluation of risk perception. The policy-maker relates to the scientist and sometimes to the sociologist, but there has been no logical attempt to bring it all together." As far as Professor Goodman was concerned, however, the seminar summarised the state of RIA art and pointed to areas where more work could usefully be done to try to shed some light on the way many social policies are made. One of these, which the Institute hopes to take up, concerns threats of small probability but high significance: how could these be incorporated into RIA? How can the management process deal with them? Another area is "safety engineering": using RIA to identify technological systems' weak spots and cost-effective methods of improving them. Then there is a need for *post hoc* and comparative risk studies. The list is long, and illustrates how little we know about working out the greatest good for the greatest number. □

**From a paper presented at the seminar by Dr Raphael Kasper of the USA's National Academy of Science's Environmental Studies Board.*

correspondence

Rothschild: an antidote to panic

SIR.—There are a number of inaccuracies or misrepresentations, doubtless unintentional, in the leading article (30.11.78) on my Dimpleby Lecture. I shall give a few examples:

● "Lord Rothschild . . . argued that we should develop a table of risks so that we could compare, say, the risk of our dying in an automobile accident with the risk of Baader-Meinhoff guerillas taking over (sic) the nuclear reactor next door." I specifically said that the risk of such a terrorist gang gaining entry to a nuclear power station was secret and *therefore unknown*.

● The leader claims I implied that viewers could not convert "probabilities from one form to another". The reverse is the case as evidenced by the visual example given, actually on a pocket calculator which was seen by the audience. The example was $109 \div 109 = 1$; $1,000,000 \div 109 = 9,200$. (Must I say, in *Nature*, that $1,000,000 \div 109 = 9,174.3119$?)

● I was said not to have quoted "a single error on the risks I enumerated". The reason was quite simple: the BBC did not like references to confidence limits or intervals, although I spent some time in the lecture on the importance of what I call "tolerances". In addition, I said that "1 in 100 is not exactly 1 in 100 in the world of risks, but, for example, *probably* somewhere between 1 in 95 and 1 in 105." If you had bothered to look at the printed version of the Dimpleby Lecture which was obtainable on 24 November, the day after the lecture was broadcast, you would have found references to confidence limits under six of the seven tables in the lecture. Ranges were given in the other one, Table 6. There are other misrepresentations which I did not expect in an editorial in *Nature*.

What is more important is that your leader-writer has failed to understand the *constraints* imposed by talking about a highly complex and technical subject to 5 million people, the overwhelming majority of whom are laymen, in contradistinction to the few thousand who read *Nature*; and the *restraints* imposed by the BBC with their undoubted expertise.

I fear I am not competent to give a lecture on "the democracy of risk assessment", whatever you mean by that phrase. Let us hope your leader writer will soon give it, if only so that we know what it is.

As stated in the last but one paragraph of the lecture (p. 19), two of its main objects were to induce people to think about risks "for a minute or two", and "to compare the different risks around us." If I may quote myself in conclusion (p. 3): "Comparisons, far from being odious, are the best antidote to panic." In the editor's case the panic appears to be about the use of numbers.

ROTHSCHILD

N. M. Rothschild & Sons Ltd.
New Court, St Swithin's Lane, EC4

SIR.—In your forceful editorial, you assert that the quantitative assessment of risks is not enough and that emotional reactions also deserve a role in decision-making. True, and indeed we live in a society where emotion is hardly likely to be under-represented in political decision-making. But emotional reactions are not necessarily and automatically decisive, and surely Lord Rothschild was merely concerned to get across the fact that such a thing as risk calculation exists and that *it* deserves a part in decision-making too. Many non-scientists don't know how risks are calculated, especially on an *a priori* basis; is it arrogant to draw attention to the fact that such calculations *can* be made?

One of the most intractable problems in the discussion of nuclear power (and genetic experiments, *et hoc genus omne*) is that the expert is the victim of a classic catch-22: he can only acquire expertise of certain kinds, it is implied, by working in the industry or science that is under attack, and that, it is asserted, automatically destroys his objectivity (and the non-objective cannot be a real expert). But if emotional bias is to be automatically presumed in the expert, then how is the undoubted emotional bias of the external attackers (*ex hypothesi*, they cannot be experts!) to be assessed? Does not that bias nullify the validity of the attack? In this connection it should be remembered that a recent public survey in Britain elicited the fact that a large majority of the public prefers to trust the technical judgment of the experts on nuclear power, intellectual prostitutes though they apparently are.

Emotional reaction to such things as nuclear reactors is a slippery basis for decision-making. I don't much like paying taxes, but I would hesitate to put that consideration too far ahead of dull calculations about the need to pay old age pensions somehow. How is allowance to be made for the fact that fears can be whipped up for reasons which quite often are largely political? Is it to be accepted that certain forms of risk are psychologically intolerable however small they are, for inscrutable reasons, whereas others are cheerfully accepted even though not negligible? For instance, it is notable that there are no public attacks on the transport of liquefied natural gas. Rothschild quoted figures implying that this process is even safer than nuclear power, yet a large LNG carrier bears explosive power equivalent to an atomic bomb. Though the LNG disaster involving a lorry in Spain indicates the destructive power of the fuel, the fact that no ship has blown up yet should not (on the analogy of the arguments applied to nuclear power) assuage the fears of those who live in harbour-towns where such ships are unloaded. Why not? Could it be that such an attack would be politically embarrassing because most LNG comes from the Third World?

Even if, for argument's sake, one postulates that no one working in the nuclear energy industry is to be trusted to give an honest opinion on contentious matters, it remains true that there exists a distinct group of experts, such as nuclear inspectors and medical radiation specialists, who can cast a cool and independent eye over some of the issues in dispute. For instance, Sir Edward Pochin, who is a medical professor, recently gave a lecture entitled "Why be Quantitative about Radiation Risk Estimates?" (National Council on Radiation Protection and Measurements, Washington, D.C., Lauriston Taylor Lecture No. 2, 1978). He makes a number of points in the special field of medical radiation protection which are very reminiscent of Lord Rothschild's and in the last part of his lecture he puts together some risk calculations from the UN, OECD, the International Commission on Radiological Protection, the US National Safety Council and the like (all, one would think, immaculately independent bodies) and concludes that the death risk from the total radiation exposure, from the entire uranium cycle, for an average citizen (including 'nuclear workers' in the average), in a country where one kilowatt electrical power per citizen comes from nuclear stations, is equivalent to the death risk from smoking one cigarette every two years. He adds that he recognises that relative acceptabilities of the two risks is another matter, but believes that "the numerical comparisons of this type do have a certain value in letting radiation be seen in a proper context as one of the numerous potentially harmful components of the occupational, as of the general, environment. . . ." The essential answer to your objection to Lord Rothschild's lecture, surely, is that it can only be beneficial to have context.

Two other brief points should be made about your editorial. Firstly, you point out the special difficulty of assessing 'unknown risks'—risks of something that has never yet happened, like a serious reactor accident, or a major LNG ship disaster. But surely it is a very odd attitude to treat, by implication, something so safe that it has never yet gone seriously wrong over a period of years, as more suspect (merely because no *historical* risk estimate is feasible) than, say, coal-mining or deep-sea fishing? This implies that the more successful an industry is in minimising fatalities (and the nuclear power industry has, to date, a unique record), the larger the part that emotion (as distinct from facts) should play in assessing its desirability. Odd!

Finally: you make the intrinsically very powerful point that one cannot quantify the risk of the loss of some civil liberties if nuclear power expands, or, for that matter, quantify the emotional reaction to that eventuality. But who shall assess the risk of major social disorder occasioned by very serious energy shortages in future if nuclear power is stopped, and what numerical value would such a disamenity carry?

That kind of argument always pulls in two directions.

Emotion is unavoidable in this kind of debate, and your readers will no doubt discern a smidgin of it in this letter. But in science, emotion should be the engine and reason should hold the steering-wheel and press the accelerator and brake. Risk analysis quite properly helps to guide the foot between those rival pedals.

ROBERT CAHN

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We did not wish to imply that emotional commitment characterised only one side of the nuclear debate—far from it—only that it was impossible to assess risks without taking such factors seriously. Further, the unknown risk referred to was the risk of failure of a commercial scale fast breeder reactor, for which there is no experience.—Ed.

Solar activity and influenza

SIR.—I would like to elaborate further on the remarkable correlation between influenza A viral antigenic shifts and peaks of the sunspot cycle as noted by R. E. Hope-Simpson (*Nature* 275, 86; 1978).

Ancient "cosmic and telluric theories" regarding influenza epidemics date as far back as the time of Hippocrates (Thomson, D. & Thomson, R. *Ann. Pickett-Thomson Res. Lab.* 9, 257–261; 1933 and 10, 1125–1140; 1934). Over half a century ago, F. G. Crookshank (*Mil. Surg.* 59, 284–290; 1926) defended the hypothesis that influenza pandemics and solar activity were correlated, and this possible relationship was further studied by M. Mygge (*Acta Med. Scand. suppl.* XXXII, 105–134; 1930). Since the discovery of a filtrable virus as the aetiological agent of influenza in the 1930s, the notion of a solar-influenza association has been largely dismissed and forgotten.

Except for the pandemic of 1889, the beginning dates of historical influenza pandemics in the 18th and 19th centuries (Mote, J. R. In: *Viral and Rickettsial Diseases*, Harvard University Press, 257–261, 1933), as well as pandemics and viral antigenic shifts in this century (Kilbourne, E. D. *J. Amer. Med. Assoc.* 237, 1225–1228; 1977), appear to have occurred in years of high sunspot number. From our present knowledge of influenza, it is not unreasonable to assume that these severe historical pandemics spreading rapidly to world-wide proportions were associated with major shifts in the influenza A haemagglutinin (H) and neuraminidase (N) surface antigens.

The mechanisms on how the solar cycle

can affect influenza pandemics may only be speculated upon at this time. The amount of ultraviolet light incident on the surface of the earth is increased in years of high sunspot activity (Pettit, P. *Astrophys. J.* 75, 185–221; 1932), and this can conceivably accelerate the mutation rate of the influenza virus. The relationship between long-term cyclical fluctuations in meteorological and climatic conditions with the sunspot cycle remains controversial at this time. It is plausible, nevertheless, that during years of high solar activity, a more favourable ecological environment may exist for the genetic recombination of human and animal influenza viruses, with the emergence of new pandemic strains.

It is interesting to note that while the swine influenza virus (HswN1) reappeared in 1976, its spread into pandemic or even epidemic proportions did not occur in this year of sunspot minimum. The resurfacing of the H1N1 subtype ("Russian flu") in December of 1977 was completely unexpected. The sunspot cycle was beginning its rapid ascent at that time.

It would not at all be surprising if a new strain of influenza A virus should emerge and dominate in the sunspot maximum years of the near future. On the other hand, perhaps what could only be expected from the elusive influenza virus is the unexpected.

ROY ING

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Seveso: premature optimism

SIR.—At the recent Seventh International Congress of Pharmacology in Paris, Dr G. Reggiani of the Hoffman-La Roche Medical Board presented a communication on the clinical features of the July 1976 TCDD accident in Seveso. The Mario Negri Institute have closely followed many aspects of the investigations since then, and we feel some comment is necessary.

TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxine) is one of the most powerful known toxic chemicals in animals, especially as regards its cumulative and delayed effects. So there cannot but be serious concern about the long term effects of the accident on man, regardless of the present impossibility of making any defensible prognosis (S. Garattini *Biomedicine* 26, 28–29; 1977).

Since the accident, Hoffman-La Roche, which owns ICMESA through its subsidiary Givoudan, has been faced with many problems, of which public relations is certainly not the least. Its representatives have with every right denied false or exaggerated press reports such as charges that ICMESA had been secretly engaged in manufacturing

warfare chemicals. After a while, though, they began to comment optimistically on the potential health risks to people living in the polluted area. For example, Dr Reggiani noted that: "In the case of TCDD man seems to respond to a certain extent differently from other animal species . . ." (J. Walsh *Science* 197, 1064–1067; 1977). There is no question that all efforts to minimise exposure of the residents in the disaster area should not be discontinued and we fervently hope Dr Reggiani's opinion proves right. But he seems to be rushing it somewhat by extending his increasingly sanguine attitude to retrospective evaluation.

His Paris communication was obviously biased by his desire to make little of the possible damage to human health. Thus he offered reassuring interpretations of findings such as those on chloracne, the typical skin lesion after TCDD exposure in humans, pointing out its "quick healing tendency" with the unsupported implication that it was quantitatively linked to exposure. He failed to mention its recurrence in people from widely scattered areas. Regardless of whether they were based on poorly reliable data such as observed rates of spontaneous abortion, Dr Reggiani confidently presented negative findings as strong evidence for the absence of TCDD induced pathology, with no criticism of the epidemiological methodology adopted or related problems deriving from the lack of adequate historical controls.

These points are evident from his abstract in the official volume (*Abstract n.* 2890, 953) which concludes: "The measures of protection of the population and prevention of further damage carried out at our request by the local authorities have avoided the severe injuries observed in previous similar accidents".

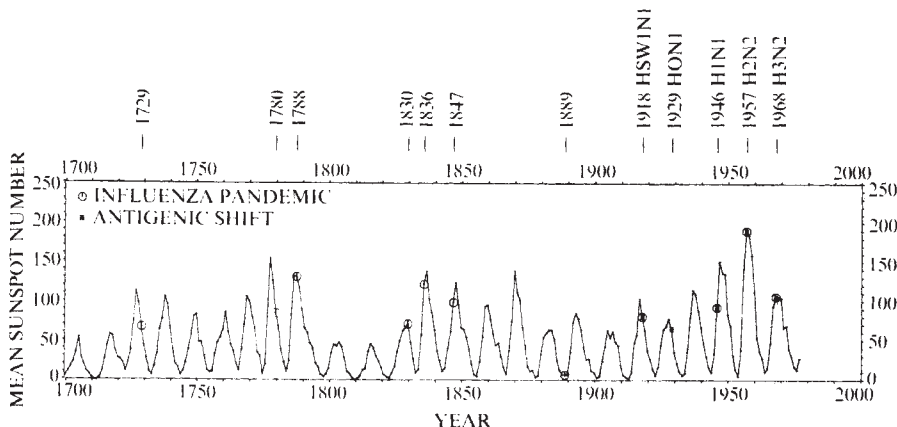
In view of this statement we must recall that the Seveso case cannot be compared with any previous accident involving TCDD because of 1) the unprecedentedly heavy contamination of inhabited areas, 2) the delay in recognising the nature of the accident and the resulting lag before protective measures were adopted, and 3) the significant amounts of TCDD persisting in the environment where people live. No mention of the TCDD risk was made to the local authorities before the disaster and even after it. Roche took almost two weeks to admit that TCDD had escaped from the plant.

It is unfortunate when someone takes advantage of a scientific meeting to present such misleading statements. People with no knowledge of the background and complexity of the Seveso case and the difficulty of epidemiological studies, may be led to accept the idea that thanks to Roche, all is well in Seveso now. This sounds outrageous to those who have already suffered distress from the disaster, apart from all considerations of feared future effects on health, which only time will reveal.

In addition and perhaps most relevant, our concern about Dr Reggiani's statement is of practical value. Everybody knows how difficult it is to motivate people to keep coming back for the controls needed for epidemiological studies. If the idea is spread around that there are no risks it will be difficult to collect long term data. No data available may well represent the best possible proof that no toxic effects occurred!

L. MANARA, S. GARATTINI

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Reflections on the occasion of my birthday, by Mr Davy

IT has been a matter of some gratification to me, in my present rather distant circumstances, to note that the Anniversary of my birth has not gone unnoticed by your esteemed Journal. I refer, Sir, to the remarkably accurate and well executed likeness of the lamp which is associated with my name, that occupies the entirety of your frontispiece. By the yardstick of eternity two hundred years is a mere nothing. It would be churlish to complain of a lack of recognition, were it not that various dabblers and meddlers have received more than their proportionate share of such credit as your transitory World offers. It is true that the Royal Institution has seen fit to mark this Anniversary with an exhibition. But why is access restricted to Tuesdays and Thursdays between the paltry hours of noon and four? And why do they charge a derisory fleabite of only twenty pence? In 1811, in Dublin, tickets cost two whole guineas to hear me lecture on 'Peculiar Observations in Electro-chemistry'. And even so all five hundred and fifty tickets were sold within a week. Subsequently I was told the self-same tickets changed hands on the street at up to twenty guineas apiece! In those days Chemistry was much more popular as a subject for lectures, than Astronomy, Mechanics, or Natural History. A critic wrote, 'These subjects are not susceptible of any brilliant exhibitions; there is no noise, no fire—and the amphitheatre never fills, but for Mr Davy. A small bit of Potassium thrown in a glass of water, or upon a piece of ice, never fails to excite a gentle murmur of applause! And the audience consisted of young Gentlemen together with an equivalent number of young Ladies of High Quality.'

I know that Dalton and Faraday have done very well since—they have Divisions named after them in the Chemical Society. But as for Dalton all that talk about atoms; it was mere speculation, no experimental evidence. As I said at the time, 'it's more ingenious than important'. And as for Mr M. Faraday, I very much resent those who say that he was my greatest discovery. Let me remind you that, whatever became of him afterwards, he was only my Assistant. He may have discovered the Laws, but I discovered the Elements: Sodium, Potassium, Calcium, Magnesium, Barium, Strontium and Chlorine. Electricity from my Giant Pile was able to exert a power on substances such as had not been exerted before. Even mighty Soda and Potash yielded themselves to sustained attack. Not all Savants could accept my

simple view of the little molten globules emerging through the crust of the potash and bursting into purple flame. This was not to be the last time that Gay-Lussac and his henchman Thenard obstructed the course of Natural Philosophy. Carried away by their isolation of Boron—a remarkable achievement for them—I may have perchance referred to Gay-Lussac as being the Head of Living Chemists in France. But their notion that my Potassium was a mere Hydride of Potash could not be sustained and can only be regarded as being vexatious. No wonder I resent Gay-Lussac's Bicentennial—200 francs a head, no half price for children and open all the week including Sundays! I grant you that M. Lussac was a competent experimentalist; somehow his numbers always fitted. But Laws are merely reflections of Man's puny attempts to describe the omnipotence of the Supreme Being:

"All things most glorious on the Earth
Tho' transient and short lived they seem,
Have yet a source of heavenly birth
Immortal,—not a fleeting dream"

And I speak of Experiments, not Laws. As I once said, 'It is more laborious to accumulate facts than to reason concerning them; but one good experiment is of more value than the ingenuity of a brain like Newton's.'

My first experiments were conducted in Mr Tonkin's house in Penzance and relied on such apparatus as wine-glasses, tea-cups, tobacco-pipes and crucibles, securing preference upon the kitchen stove to the more ordinary domestic comestibles. I shall always be beholden to the generosity of Dr Thomas Beddoes and the Pneumatic Institute of Bristol but it was at the Royal Institution, following the work of Nicholson and Carlisle, that I demonstrated that Hydrogen and Oxygen alone were the true products of galvanic action on pure water. I discovered to my manifest astonishment that the two gases could be separated if two gold plates, dipped into separate vessels of water were linked by the fingers of my own hands. This was not the first time that my body had been used as a tool in the Laboratory. And, a fig, Sir, for your Health and Safety at Work Act! In 1806 I demonstrated the relation of electricity to chemistry using three vessels connected by moist fibres. In front of the very eyes of an audience, I immersed metal plates in two outer vessels containing Barium Nitrate and Sodium Sulphate and these outer vessels were linked each to the other through a third vessel containing pure water. The action of the Giant

Pile on this apparatus brought forth a magnificent deposition of solid white Barium Sulphate in the middle vessel. Such a spectacle could only be interpreted as a manifestation of Nature's innermost Secrets. For the first time I realised that some substances were attracted and some repelled by the quality of the electrification of the plates. I declared 'These attractive and repulsive forces are sufficiently energetic to destroy or suspend the usual operation of Chemical Affinity'. One cannot speculate with profit on the nature of electricity, but I had no doubt it was connected with Chemical Affinity and the very essence of Matter.

The Lamp you have, Sir, in your frontispiece, should not be the only reminder to your Readers of the festive Season. Why, Sir, on Boxing Day—aptly named—in 1799 I discovered the medical properties of Nitrous Oxide. I enclosed myself in an airtight breathing box, having a cubic capacity of about nine and a half cubic feet. I was in the box for one and a quarter hours, and breathed over twenty quarts of pure nitrous oxide. A thrilling extending from the chest to the extremities was almost immediately produced. I felt a sense of tangible extension, highly pleasurable in every limb; my visible impressions were dazzling and apparently magnified. By degrees as the pleasurable sensations increased, I lost all connection with external things; trains of vivid visible images rapidly passed through my mind and were connected with words in such a manner, as to produce perceptions perfectly novel. I existed in a World of newly connected and newly modified ideas. I theorised; I imagined that I made discoveries. And that is why, Sir, I crave the courtesy of your columns to enquire why I should be remembered only by my Lamp (as if I was Miss Nightingale) rather than as the Father of British Anaesthesia. I need hardly remind you that the Poet Southey wrote 'Davy has invented a new pleasure for which language has no name. I am going for more this evening, it makes me strong and happy! So gloriously happy! Oh, excellent air-bag!' And in 1801 Wordsworth and Coleridge beseeched me to teach them Chemistry in a special Laboratory to be constructed at Keswick. Had I acceded to their request and gone to the Lake District, rather than the Royal Institution, the benefits to English Literature and to Chemistry remain to this day, Sir, incalculable.

John Albery and Peter Barratt, of Imperial College, claim to have found this letter.

news and views

Intervening sequences in the mitochondrial genome

from Giorgio Bernardi

SINCE its foundation by Boris Ephrussi 30 years ago, mitochondrial genetics has made great advances and yet this subject has remained an area of interest to only a small number of laboratories. Whatever the explanation for this, the situation is not likely to last much longer since exciting results recently obtained in one particular system, the mitochondrial genome of yeast, should stimulate very wide interest.

The mitochondrial genome of *Saccharomyces cerevisiae* comprises about 100 identical, circular, supercoiled DNA molecules per diploid cell. Each genome unit is about 50 million in molecular weight, a value five times greater than that of the mitochondrial genome of organisms as far apart in terms of evolution as *Chlamydomonas* and man, but smaller (by a factor of almost two) than that of higher plants. Each genome unit is known to encode two ribosomal RNAs, at least 30 tRNAs, and nine proteins: three of the seven subunits of cytochrome *c* oxidase, one of the seven subunits of the *bcl* complex, three of the ten subunits of the ATPase complex, and one protein, *var1*, of unknown function. The other mitochondrial proteins are specified by nuclear genes, a fact which emphasises the very close cooperation between nuclear and mitochondrial genes in mitochondrial biogenesis and function.

The fivefold difference in unit size between the mitochondrial genomes of yeast and, say, man, is difficult to explain in terms of coding potential since it is unlikely that the former codes for so many more gene products than the latter. Put in a different way, the discrepancy between the unit size of the mitochondrial genome of yeast, about 50 million, and the amount of DNA

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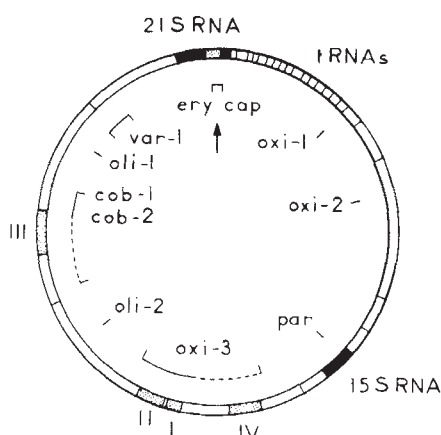


Fig. 1 The mitochondrial genome of *S. cerevisiae* modified from Borst & Grivell Cell (in the press). The location of the genes for 21S and 15S RNAs is indicated. Thin, radial lines indicate the position of tRNA genes. Roman numerals indicate genome segments absent in *S. carlsbergensis*. The 21S RNA insert is represented by the dotted region splitting the 21S RNA gene. The approximate location of a number of genetic markers as well as the *cob* loci referred to in the text is indicated inside the ring. The arrow indicates the origin of the map which corresponds to a *SalI* site.

required to encode its known gene products, about 10 million, raises the same problem as the so-called 'c-value paradox' of the nuclear genome of eukaryotes, namely, what is the role of the 'excess DNA'? An answer to this problem is emerging from two different experimental approaches.

A direct molecular approach revealed, some years ago, that half of the mitochondrial genome of yeast consists of AT spacers (GC<5%) formed by short, repeated dAT:dAT and dA:dT sequences. The other half, presumed to correspond to the structural genes and to their regulatory elements, is extremely heterogeneous in base composition (since it ranges from 21% GC for ribosomal genes, to about 35% GC for tRNA genes, to 65% GC for GC-rich segments representing 10% of the

genome) and contains 60–70 clusters of the sequences CCGG and GGCC¹ which are palindromic and homologous in sequence^{2,3}, as originally postulated by Prunell and Bernardi. By cutting these clusters with the restriction enzyme *HaeIII*, the genome can be split into 60–70 fragments, most of which seem to be formed by a GC-rich region, a gene (or gene segment, see below), and a spacer. Interestingly enough, the *HaeIII* fragments vary in length and (to some extent) in number in different strains, two factors which are responsible for a variation of ± 5 in the size of the corresponding genomes⁴.

The functions of the spacers, of the (CCGG, GGCC) clusters and of the GC-rich segments are still a matter of conjecture. The presence in them of homologous or quasi-homologous sequences appears, however, to be responsible for the high frequency of recombination phenomena which characterise the mitochondrial genome of yeast. Unequal crossing-over events seem to account for the changes in length of *HaeIII* fragments, which accompany genome evolution⁴ and genome recombination in sexual crosses⁵ as well as for the excision of genome segments leading to the defective genomes found in spontaneous 'petite' mutants⁶.

1. Prunell & Bernardi *J. molec. Biol.* **86**, 825 (1974); **110**, 53 (1977).
2. Macino & Tzagoloff *Proc. natn. Acad. Sci. U.S.A.* (in the press).
3. Cosson & Tzagoloff submitted to *J. biol. Chem.*
4. Prunell *et al.* *J. molec. Biol.* **110**, 17 (1977).
5. Fonty *et al.* *J. molec. Biol.* **119**, 213 (1978).
6. Fonty *et al.* submitted to *J. molec. Biol.*
7. Tzagoloff *et al.* *J. Bact.* **250**, 826 (1975).
8. Slonimski *et al.* in *Biochemistry and Genetics of Yeasts* (eds Bacila *et al.*) (Academic, 1978).
9. Mahier *et al.* *ibid.*; Linnane *et al.* *ibid.*; Haid *et al.* submitted to *Eur. J. Biochem.*
10. Borst & Grivell Cell (in the press).
11. Bos *et al.* *Nature* **275**, 336 (1978).
12. Faye *et al.* *Molec. gen. Genet.* (in the press).
13. Hahn *et al.* submitted to Cell.
14. de Vries & Kroon *Neurospora Newsletter* No. 25.
15. Sanders *et al.* *Molec. gen. Genet.* **157**, 239 (1977).

The starting point for the genetic approach was the discovery of the so-called *mit*⁻ mutants in which mitochondrial functions are impaired by point mutations or small deletions affecting the mitochondrial genes coding for the subunits of respiratory enzymes or of ATPase. Mapping work in several laboratories has revealed that both the *cob* (or *box*) region, coding for apocytochrome *b* (the mitochondrial subunit of the *bcl* complex), and the *oxi-3* locus (which codes for subunit I of cytochrome *c* oxidase) correspond to DNA segments several times larger than expected (see Fig. 1). More recent work⁸ indicates that non-coding regions and regulatory sequences are present within the *cob* region. The data of Slonimski *et al.*, for instance, indicate that mutants lacking functional cytochrome *b* map in seven discrete clusters dispersed over 6,000–10,000 base pairs. Mutations at non-adjacent loci 4/5, 1 and 6 fall into a single complementation group and produce abnormal apocytochrome *b*. On the other hand, mutations at loci 2, 3 and 7 complement mutations at loci 4/5, 1 and 6, complement each other, and present a disturbed synthesis of both subunit I of cytochrome *c* oxidase and of cytochrome *b*. Most of these pleiotropic mutants are temperature-sensitive and accumulate multiple 'new' mitochondrial translation products. The genetic and biochemical work just mentioned is further supported by electron microscopic work⁹ on hybrids between the 18S mRNA transcribed from the *cob* region and restriction fragments from the same region; this shows that the gene for cytochrome *b* consists of at least five coding regions separated by four non-coding regions which are between 600 and 2,000 base pairs long.

At least one other mitochondrial gene, the gene for the 21S RNA, is also split, in addition to the two already mentioned. Electron microscopy of 21S RNA with a restriction fragment containing this gene, show that this contains a 1000 base pair insertion which is closely correlated with the ω^+ allele, a polarity locus affecting the transmission of neighbouring genetic markers in crosses^{10,11}. Strains having the ω^- allele lack the insertion, which is always transmitted in $\omega^+ \times \omega^-$ crosses to the ω^+ progeny. Very interestingly, a large intervening sequence¹² containing tRNA genes^{13,14} has been found in the gene for the 24S RNA of *Neurospora* mitochondria.

An important question raised by these findings concerns the relationship of intervening sequences with the sequence elements (spacers and GC-rich segments) discussed above. More specifically, it may be wondered whether some of the homologous sequences present in those elements are

not used in the RNA splicing. While current biochemical and genetic work will certainly lead to an answer to this question, it is noteworthy that the large regions of strain-dependent genome variability are correlated with the presence of long dA:dT sequences and map at the *cob* and *oxi-3* regions (Fig. 1). Finally, it should be noted that while the presence of split genes further weakens the hypothesis of the prokaryotic origin of mitochondria, its main interest undoubtedly resides in the fact that intervening sequences have now an easy genetic handle which should allow us to gain a better understanding of these puzzling genome elements. □

Enzyme defects and immune dysfunction

from Fred S. Rosen

GENETIC deficiency of two enzymes in the purine degradation pathway, adenosine deaminase (ADA) or nucleoside phosphorylase (NP), results in fatal immunodeficiency disease. E. Giblett (University of Washington, Seattle) discovered ADA deficiency serendipitously while examining erythrocytes for informative enzyme polymorphisms from a child with severe combined immunodeficiency (SCID), a profound deficiency of T and B lymphocytes. She subsequently discovered NP deficiency in a child with severe T cell deficiency. These are the only genetically determined immunodeficiencies that have a known biochemical basis. Although ADA and NP are distributed in all mammalian tissues, only the lymphoid system is affected by the deficiencies.

A meeting was held recently at the Ciba Foundation* to discuss the biochemical consequences of ADA and NP deficiency, particularly as to how they affect the ontogeny of lymphocytes.

Both ADA and NP deficiency are inherited as autosomal recessive phenomena. ADA has been assigned to chromosome 20 and NP to chromosome 14. ADA deficiency results in a more profound immunodeficiency than does NP deficiency. Of all infants affected with the autosomal recessive form of SCID, 35 to 50% have ADA deficiency. NP deficiency is known in only six kindred. Affected children in these six kindred have a severe A cell deficiency, normal immunoglobulins, increased antibody responses, autoantibodies, and poor *in vitro* responses to

mitogenic stimuli (A. Ammann, University of California, San Francisco). By contrast, ADA deficiencies have a profound defect of both T and B cells and no *in vitro* or *in vivo* immunological function. Although the erythrocytes are completely deficient in ADA, a mutant enzyme has been found in fibroblasts and lymphocytes in low amounts (approximately 15% of normal). This has enabled the prenatal diagnosis of this disease in amnion fibroblasts (R. Hirschhorn, New York University, New York).

It was found that NP deficient cells accumulate 2'-deoxyguanosine triphosphate (dGTP) in erythrocytes, and lymphocytes whereas ADA deficient cells have abnormally high levels of 2'-deoxyadenosine triphosphate (dATP) in these cells (A. Cohen & D. W. Martin, University of California, San Francisco). D. A. Carson (Scripps, La Jolla) showed that preferential T cell trapping of deoxyadenosine and deoxyguanosine and subsequent accumulation of dATP and dGTP is due to the high kinase content of T cell lines and their relative poverty of 5'-nucleotidase. L. Thelander (Karolinska Institute, Stockholm) obtained calf thymus ribonucleotide reductase in high purity. The allosteric regulation of this enzyme requires 1×10^{-3} M ATP as a positive effector, and is readily inhibited by dATP and to a lesser extent by dGTP and dTTP. dCTP has no inhibitory effect. In fact, dCDP can rescue murine mutant S49 lymphoma cells that have ADA and/or NP deficiency. N. M. Kredich (Duke University, Durham) offered a very reasonable explanation for the greater severity of ADA deficiency in that adenosine accumulation inhibits the hydrolase of S-adenosylhomocysteine. This substrate accumulates and clogs the methyl donor activity from S-adenosylmethionine. Thus DNA synthesis in ADA⁻ lymphocytes is obstructed at the ribonucleotide reductase step and DNA methylation is inadequate.

Establishment of lymphoid chimaerism with transplants of histoidentical marrow has been the most satisfactory therapy for ADA deficiency. Lacking a bone marrow donor for an ADA deficient infant, S. H. Polmar (Case Western Reserve, Cleveland) used frozen human erythrocyte transfusions as a source of exogenous ADA. This succeeded in reducing dATP levels in lymphocytes and inducing normal immune function. This procedure was extended with further success to an NP deficient child (B. J. M. Zegers, Wilhemina Kinderziekenhuis, Utrecht). W. N. Kelley (University of Michigan, Ann Arbor) has obtained both ADA

*Enzyme Defects and Immunity Dysfunction, held 7–9 November. The Proceedings of the conference and the discussion will be published in their entirety for the Ciba Foundation by Excerpta Medica, Amsterdam.

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and tissue ADA binding factor in a high state of purity. Purified ADA awaits trials of its clinical usefulness. Also, on theoretical grounds, deoxycytidine may have therapeutic benefits for NP and ADA deficient.

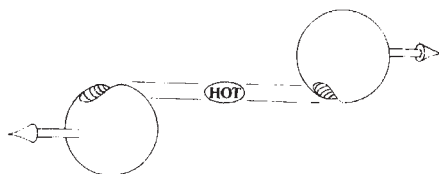
J. F. Smyth (Royal Marsden Hospital, Sutton, Surrey) has infused terminal cancer patients and patients with T cell acute lymphocytic leukaemia with deoxycytidine, a potent ADA inhibitor. He achieved striking lymphocytocidal effects. Potent immunosuppressive and oncolytic agents may emerge from the study of these rare immunodeficiencies which result from a failure of purine breakdown. □

Multipion production in relativistic heavy-ion collisions

J. M. Irvine

In recent years the nuclear physics community has been excited by the possibility of new accelerator systems capable of producing relativistic heavy-ion beams. There has been theoretical speculation for several years about using such beams for producing new, exotic nuclear phenomena such as superheavy elements, nuclear shock waves, pion condensates (the nuclear analogue of a ferromagnetic phase transition) and quark matter.

If one fires high energy nucleons at one another the most obvious products are pions, and one of the first things to be understood about relativistic heavy-ion collisions then is the pion production. Earlier this year (Fung *et al. Phys. Rev. Lett.* **40**, 292; 1978) data became available from the Bevalac at Berkeley on negative pion production in relativistic heavy-ion collisions from beams of ^{40}Ar at 0.4, 0.9 and 1.8 GeV/nucleon and ^{12}C beams at 0.4 and 2.1 GeV/nucleon on targets of LiH , NaF , BaF_2 and Pb_3O_4 . As was to be expected theoretical papers aiming to interpret the data have followed thick and fast. The simplest picture (Vary *Phys. Lett.* **40**, 295; 1978) considers that the colliding nuclei are moving so much faster than the internal velocities of the nucleons of which they are composed that this internal motion can be ignored and the heavy ion collision can be viewed simply as a large number of independent nucleon-nucleon collisions. Vary's calculations predict too many events with high negative pion multiplicities, that is, events where more than 15 negative pions are produced, and attempts to correct this feature by allowing the pions to interact with the



nucleons to produce baryon resonances have not been successful. A better account of the pion multiplicities is provided by the fireball model (Gyulassy & Kauffman *Phys. Rev. Lett.* **40**, 298; 1978). In this model, as the target and projectile sweep by each other, the material in the geometrical overlap is swept out and assumed to be at rest in the centre of mass frame. The enormous initial kinetic energy is assumed to be converted into internal thermal energy. The pions are assumed to be produced in this extremely hot fireball fragment (see Fig. 1). Gyulassy and Kauffman conclude that this model is adequate to explain the pion multiplicity data and come to the conclusion that the independent nucleon-nucleon model predicts too many high multiplicity events because it ignores pion absorption. They go on to assert that

there is a very large class of dynamical models which will give results identical to those of the fireball model: the only requirements on the models being that the pions are uncorrelated and that the average pion multiplicity is correctly given.

This assertion seems to be supported by recent results of Afek *et al.* (*Phys. Rev. Lett.* **41**, 849; 1978) using the collective tube model. This model uses a geometrical picture of the heavy ion collision like the fireball model and considers the collision of two 'tubes' of nucleons in the target and projectile nuclei. In the centre of mass frame the colliding tubes are Lorentz contracted into narrow disks and the model then assumes that the tube-tube collisions resemble elementary particle-particle collisions. The agreement between the predictions of this model and the data of Fung *et al.* is extremely impressive. Afek *et al.* go on to claim that the agreement between the predictions of the colliding tube model and statistical thermodynamic models, like the fireball model, is entirely accidental and will disappear at higher energies for heavy projectile-heavy target systems. □

Sinks and sources of plant nutrients

from Peter D. Moore

COMMUNITIES of emergent aquatic plants are known to be among the most productive found in temperate latitudes. Dykijora (*Photosynthetica* **5**, 329; 1971) examined three such littoral plants, *Phragmites communis*, *Typha angustifolia* and *Scirpus lacustris* and obtained values for their productivity (means for the whole growing season) of up to 25, 39 and 21 $\text{g m}^{-2} \text{ day}^{-1}$ respectively. In part these high values can be explained in terms of the structure and arrangement of leaves; leaf area index is high for all these species and all possess underground rhizomes which act as storage organs and which permit rapid leaf production at the commencement of the growing season. A further factor which contributes to the high productivity of swamp habitats is the normally adequate water supply and the frequently rich provision of nutrients in the water flow.

Tropical swamps also have very high productivity. For example, Thompson (in *The Nile, Biology of an Ancient River* (ed. Rzoska) Dr W. Junk, The Hague; 1976) has estimated that papyrus (*Cyperus papyrus*) swamps in

East Africa achieve values of $35 \text{ g m}^{-2} \text{ day}^{-1}$. As with their temperate counterparts, these plant communities also place high nutrient demands upon their environment and accumulate these nutrients within their living biomass and in the peat which develops from the undecomposed litter.

Gaudet (*Ecology* **58**, 415; 1977) has measured the nutrient uptake capacity of papyrus and has obtained the following values ($\text{g m}^{-2} \text{ yr}^{-1}$) N, 103; P, 8; K, 130. These figures are similar to published data concerning *Typha latifolia* for P and K, but are twice as high for N. Such very high accumulation rates have subsequently led Gaudet to consider the value of such papyrus communities for the extraction of nutrients and hence the reduction of eutrophication in East African lakes. Nutrient cycles being what they are, however, much of the NPK build up in the biomass is released in litter decomposition. Gaudet has measured the rate of nutrient release, both through decomposition and through the direct leaching of living tissues and has estimated the proportion of the annual nutrient uptake which would enter swamp peat. The figures he obtained are N, 63%; P, 1% and K, 1%. Thus most of the P and K are released back into the environment,

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but much of the nitrogen is taken out of circulation in the peat deposit. As a system for nutrient extraction from eutrophic waters, it might be more efficient to harvest the emergent biomass periodically, but not so frequently that it would result in the undue depletion of rhizome resources. Over-cropping is known to weaken some temperate emergent plants; Conway (*J. Ecol.* **30**, 211; 1942) demonstrated such an effect in *Cladium mariscus*, which is reduced in its growth vigour if harvested more frequently than once every four years.

Just as the development of a peatland habitat acts as a sink for the major plant nutrients, so the destruction of a peatland often results in the flushing of such elements back into the environment. Such has been Israel's experience in the Hula Basin to the north of the Lake of Tiberias (Sea of Galilee). Here, extensive swamplands dominated by *Phragmites* have been drained in recent years and the peaty soils have produced rich agricultural land. As in the fens of eastern England, however, there has been a subsidence of the surface, amounting to about 10 cm yr⁻¹, as a result of the drying and contraction of the peat and its gradual

oxidation.

Avnimelech (2nd INTECOL Congress, Jerusalem, 1978) reported a further and more serious problem in the release of large quantities of nitrate from the wasting peat. It is estimated that 20,000 tons of nitrate nitrogen may be released from the area and up to 5,000 tons yr⁻¹ have been carried by the Jordan into Lake Tiberias. Such eutrophication, it is feared, could influence the local fishing industry.

Attempts have been made to control floodwater from the Hula Basin, for the greatest movements of nitrate downstream are associated with floodwater. But the most successful measure adopted so far has been the encouragement of denitrification. Alternate wetting and drying of the peat stimulates microbial denitrification and the use of sprinkling has now reduced nitrate leaching into drainage waters by 50%. One of the main crops on the peatland is alfalfa, which is a surprising choice in view of its symbiotic nitrogen fixation. The use of a nitrogen demanding crop species such as rice would seem more appropriate from a nutrient cycling point of view, but is currently precluded by economic considerations. □

More on X-ray bursts

from A. C. Fabian

SPRING and autumn are the seasons of the rapid burster. This object produces up to several thousand X-ray bursts per day over intervals of 2-6 weeks in March/April and September/October. Its location near the Galactic Centre means that each burst, of but a few seconds duration, emits in X-rays as much energy as the Sun emits in all wavelengths in one day. Large X-ray detectors on the first High Energy Astronomical Observatory (HEAO-1) were pointed at the rapid burster on 31 March of this year, yielding the light curves shown on page 587 of this week's *Nature*. A collaboration of experimenters from Massachusetts Institute of Technology, University of California at San Diego and Goddard Space Flight Center have found evidence for spectral changes in the decay of rapid bursts.

A spectral softening is observed, but to a less marked extent than appears in other X-ray burst sources, where the burst interval may be many hours. The softening in these other sources may be interpreted as black-body cooling (see for example Swank *et al. Astrophys. J.*

Lett. **212**, L73-L76; 1977), leading, with some assumptions as to the luminosities, to a measure of the size of the emitting region. This seems to be relatively constant and is consistent with the surface area of a neutron star (van Paradijs *Nature*, **274**, 650-653; 1978). The region responsible for the optical bursts seen coincident with bursts from MXB 1735-44 (Grindley *et al. Nature*, **274**, 567; 1978) is considerably larger.

There is at yet no detailed theoretical model of the rapid burster, although accretion instabilities in matter flowing onto a neutron star seem a likely candidate for the basis of any such model. The 6 month interval between periods when the burster is observed has also yet to be explained. SAS-3 observations to be published next January (Marshall *et al. Astrophys. J.* in the press) show that a trend in bursting behaviour occurs over the period for which the source is visible. This may imply that the 6 month timescale is intrinsic to the source and not, for example, due to obscuration by a binary companion.

Thermonuclear flashes near the surface of an accreting neutron star have been proposed as the source of the X-ray bursts that are observed to

Faint images revealed

from F. G. Smith

THE modern generation of optical astronomers now rely heavily on television and photon-counting techniques, so much so that photography seemed to be rapidly going out of date despite the introduction of sensitive fine grain films such as Kodak IIIa. Competition is, however, a powerful stimulus, and there are now some new photographic techniques giving astronomical photographs which cannot yet be equalled in sensitivity and detail by any new electronic device.

The first step was the hypersensitising of emulsions by soaking in dry nitrogen followed by hydrogen gas. This technique, which gives a large improvement in quantum efficiency, is routinely used in the southern sky survey at the UK Schmidt telescope in Australia. The second step is surprisingly simple, and involves only the method of taking contact copies from the new sensitised plates.

D. F. Malin of the Anglo-Australian Observatory, observed that the photographic grains in faint images are concentrated in the upper layers of the emulsion, while the background fog is distributed throughout the whole depth. He found that contact printing with a diffuse light could be arranged only to reproduce the upper layer, and thereby enhance the signal-to-noise ratio of faint images. He describes the techniques in a note on page 591 of this issue of *Nature* and shows some remarkable results on photographs of the extragalactic nebula Centaurus A and the supernova remnant in Puppis. Faint extensions of both objects are revealed for the first time.

These results are exciting for astronomy, and most gratifying as a further example of a widely useful technical advance stimulated by the needs of pure research.

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recur on intervals of hours. The flashes are due to a thermal runaway in the helium burning layer of accreted matter. Numerical computations of this process have recently been published by P. Joss (*Astrophys. J.* **225**, L123-L127; 1978) and demonstrate that X-ray bursts can be produced that bear a striking resemblance to the ones observed. The flashes occur at such a depth that heat is transported out fast enough by radiative diffusion that X-ray temperatures are achieved at the photo-

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sphere. Joss' model did not produce flashes if the temperature of the core was too high, although the core was still in thermal equilibrium with the time-averaged heat flow. X-rays are, of course, produced more efficiently by gravitational energy release when the matter is first accreted. The ratio of accretion luminosity to thermonuclear luminosity L_a/L_n must be ≥ 30 , for conventional neutron star models, and ~ 300 for Joss' model. $L_a/L_n < 25$ for MXB1759-29 and is ≤ 2 for the rapid burster, if it is assumed that the accretion luminosity emerges as relatively continuous X-rays over the same time interval as that on which bursts are observed. This tends to rule out thermonuclear flashes for the rapid bursts and may prove to be a problem for this model in general. Future work on X-ray bursts will one hopes improve our knowledge of neutron stars and thereby teach us some high energy physics. $\square$

Gene conversion in mitochondria

from D. H. Williamson

THE article on page 577 of this issue by Strausberg and his colleagues from Dallas will rekindle interest in a long-standing problem in the mitochondrial field, namely the mechanism of recombination between mitochondrial DNA molecules.

Mitochondrial genetics is dominated by work on bakers' yeast, and has been ever since the discovery in this organism, some 15 years ago, of the first mitochondrial point mutations conferring resistance to either chloramphenicol or erythromycin. These drugs block protein synthesis on mitochondrial ribosomes, and it happened that these first resistant mutants all mapped close together in the region we now know specifies the 21S mitoribosomal RNA. These mutants were the first tools with which mitochondrial recombination in crosses could be studied, and quite early on, a puzzling phenomenon emerged; in certain crosses, a marked polarity was observed amongst the recombinants, alleles carried by one parent being greatly favoured at the expense of those represented by the other. This polarity was not observed in all crosses, and it was soon realised that any strain could formally be categorised as carrying a hypothetical determinant called omega (ω) in one or other of two configura-

tions, ω^+ and ω^- . Polarity of recombination was observed only in $\omega^+ \times \omega^-$ crosses, and not in either of the other two pairwise combinations. The ω determinant was independent of the mating type of the cell, but an early idea was that its role was a sexual one analogous to Hfr in bacteria and, for a while, the notion of mitochondrial sex was in vogue. However, when mutants in other unlinked genes were discovered, making four-point crosses feasible, it became clear that the analogy with bacterial sex was invalid, and in 1974 the Gif group (Dujon, Slonimski & Weill *Genetics*, **78**, 415; 1974) proposed a comprehensive model to account for all the facts then known. Essentially their model supposes that the mitochondrial DNA molecules in pairs of mating cells undergo repeated rounds of recombination. At the level of the individual molecules, recombination events are considered always to be non-reciprocal. However at most loci, gene conversion can go randomly in either direction, thus generating equal numbers of reciprocal recombinant types in the population as a whole, and obscuring the non-reciprocal nature of the events at the molecular level. At the ω locus, on the other hand, the conversion is seen as always occurring in one direction, effectively favouring alleles of the ω^+ parent, and at the same time unmasking the asymmetric nature of the molecular interactions.

We have since learnt that as far as the events at the ω region are concerned, this model is broadly correct. It has recently been shown that the 21S RNA gene of ω^+ strains carries an insertion of about 1,000 base pairs which is absent from ω^- strains. It is this insertion that seems to be responsible for 'directing' the conversion events in its immediate vicinity. Until now, however, no comparable phenomenon had been discovered in other regions of the genome, and the events at ω were coming to be regarded as something of an odd-ball.

In the light of today's article from the Dallas group, this view becomes a shade less tenable. This group reports on a rather similar state of affairs affecting a recently discovered mitochondrial gene called *var1*. This gene owes its name to the fact that its polypeptide product varies in size in different strains, the extent of this variation amounting to some 4,000 daltons, or around 10% of the polypeptides' molecular weight. The work on *var1* is of unusual technical interest, since its function is completely unknown, and all the genetic analyses have revolved round the ingenious application of gel electrophoresis to bulk mating populations. With the aid of a variety of genetic and physical mapping pro-

cedures, the Dallas group have already precisely located the *var1* gene on the map, and have shown that the altered sizes of the variant forms of the polypeptide directly reflect changes in the size of the structural gene itself.

The current paper reports on the behaviour of different *var1* alleles in crosses. It shows, rather clearly, that alterations in the size of the gene (and hence of its polypeptide product) arise as a result of asymmetric gene conversion events involving two small DNA segments (about 36 and 57 base pairs long) which can be independently inserted, by recombination, into alleles which lack them. The very demonstration of these events in a system in which phenotypic expression is restricted to the rate of migration of protein molecules in a gel, is something of a *tour de force*, and fully deserves our admiration. However, the observations have a wider significance in that the phenomena look rather similar to those seen at the ω region. In both cases the gene conversion events occur at high frequencies, and both regions possess insertions presumably responsible for 'directing' the non-reciprocal recombination events at the locus, thereby making them detectable in the overall molecular population.

The main conclusion is clear. Gene conversion is not restricted to the ω locus, and its occurrence at other regions of the genome may have been missed before now simply because of the lack of strategically placed pairs of markers to make it visible. Although no other protein products have been observed to behave like *var1*, there is no lack of evidence for other insertions in the genome (see the Strausberg article for references). Nor is there any lack of models embodying gene conversion to explain some unresolved problems in the transmission genetics of this system. Evidence such as that presented on page 577 begins to invest such models with an air of respectability. $\square$

The first division

from Clive Lloyd

PROTOPLASTS prepared from tobacco leaves now form the staple experimental diet of investigators studying viral infection, somatic hybridisation, wall synthesis, properties of the plasma membrane—the list goes on—but the intriguing fact goes almost unnoticed that in the process of removing the cell wall, non-dividing leaf cells are stimulated to divide. For instance, remove palisade cells from tobacco

leaves and few will divide in culture, but remove the wall from such cells with degradative enzymes and about half of the protoplasts go on to divide Nagata & Takebe, *Planta* **92**, 301; 1970), (although it is a common observation that protoplasts must first resynthesise a cell wall before division takes place). It would seem from this that some assembly at the cell periphery is controlling cell division in such a way that its perturbation by enzymes unlocks the potential for growth. This will come as no surprise to animal cell biologists who will be reminded of the ability of some proteases to tweak density-inhibited fibroblasts into another round of division, but compared to animal cells, the role of the cell periphery in controlling cell division has been little studied in plants. A major barrier to studying the plant cell surface with techniques used for animal cells is the cell wall itself; it prevents easy access to the plasma membrane of probes such as proteases, lectins and antibodies, which have helped outline the regulatory role of the cell surface in the division of lymphocytes. However, some recent papers hint at potential ways in which the problem can be tackled using protoplasts and a recently adopted wall-inhibitor.

Schilde-Rentschler (*Planta* **135**, 177; 1977) reported her work on the role of cell wall formation in the ability of tobacco protoplasts to form callus, finding that the addition of a cellulase preparation to tobacco mesophyll protoplasts inhibits their division. Enzymes used to degrade cell walls are notoriously—and perhaps necessarily—crude preparations, so it is difficult to pin-point cellulose deposition as a prerequisite for division when so many other sites are probably affected simultaneously. However, by adding the disaccharide, cellobiose (believed here to be inhibiting cellulase activity), to protoplasts cultured in the presence of the enzyme mixture, Schilde-Rentschler was able to restore to protoplasts the capacity to divide, thereby claiming a direct involvement for cellulose formation in allowing division to take place. It is unclear from this whether cellulose biosynthesis triggers cell division or whether cellulosic wall formation simply provides the mechanical framework which allows the division of protoplasts stimulated to do so during the earlier event of wall removal. A series of papers from Meyer *et al.* favours the latter possibility, suggesting that wall formation, although required for organised

cytoplasmic division, may be unnecessary in the events which lead to nuclear division.

To get protoplasts to divide at all in culture was, originally, far from straightforward and most workers use variations of media found successful for tobacco leaf protoplasts. Unlike such media which osmotically cushion the fragile protoplasts with sugars, Meyer & Abel (*Planta* **123**, 33; 1975) used instead, salts for maintaining osmotic strength. In this alternative system, tobacco protoplasts regenerate only a pseudo-wall which differs from wall regenerated in sugar medium by being discontinuous (patches of wall-less membrane exist which permit cell fusion in the presence of polyethylene glycol), non-rigid (does not resist plasmolysis) and lacking in some typical wall enzymes. However, the regeneration of such a pseudo-wall still permits a form of cell division to continue for 2-3 rounds. Cytoplasmic division can, therefore, take place in the absence of a rigid cell wall, but related papers (*Planta* **125**, 1; 1975; *Planta* **142**, 11; 1978) showed that the quality of those divisions is abnormal. In more normal circumstances, cells would divide by coalescence at the equator, of vesicles containing materials which go to form the separating wall; but with reduced competence to form wall in the salts medium, the empty vesicles fuse and perforate the daughter cells in a manner likened to the budding of yeasts.

Now, in their most recent paper, Herth and Meyer (*Planta* **142**, 253; 1978) go one step further and prevent even pseudo-wall formation by using a reversible inhibitor of cellulose formation 2,6-dichlorobenzonitrile. In this case, no cytoplasmic division is observed, but nuclear division continues. In fact, Meyer and Herth report that within 6 days of culture, as many as 90% of the protoplasts have performed at least one mitosis, forming, two-, four-, and multi-nucleate cells. The fact that nuclear division occurs in the presence of this inhibitor not only confirms that nuclear and cytoplasmic division are separable, but that the sequence of events leading to nuclear division continues to operate in the absence of extensive deposition of cellulose. The mitotic activity of wall-less protoplasts has been reported previously by several authors, but the potential interest of the present system is that the wall-less state can be maintained by the presence of inhibitor, thereby allowing any possible role of the plasma membrane in regulating mitosis to be examined. Further quantitative data on the contribution that nuclear division, as opposed to cell fusion, makes to the polynuclear state,

would be welcomed, as would a greater understanding of how the inhibitor works. Whether or not these particular experiments stand the test of time, their general motive seems right: that we should seek novel approaches to investigate the role of the cell surface in regulating plant cell growth. □

Can magnetic order and superconductivity coexist?

from B. R. Coles

It has long been recognised that in rough approximation magnetism and superconductivity are sworn enemies. This is shown not only in the ability of an applied magnetic field to destroy the superconductive state but also in the strong suppression of T_c , the transition temperature, by additions of magnetic-moment bearing atoms, where simple solutes have little effect. When enough magnetic atoms have been added to destroy superconductivity it is often succeeded at low temperatures by a state in which the solute moments couple by way of the (now normal) conduction electrons. In random solid solutions the resultant state cannot easily be one of long-range magnetic order—it is now generally called a spin glass but there have recently been discovered some atomically well-ordered intermetallic compounds containing a fraction of moment-bearing atoms (rare earths with partly filled 4f electron shells) where tendencies towards magnetic order and towards superconductivity are both present. These are compounds of the form RE Mo₆S₈ (Fischer *et al.* *Solid State Commun.* **17**, 721; 1975)—the Chevrel phases—and of the form RE Rh₂B₄ (Matthias *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 1334, 1336; 1977) where RE is a rare-earth metal. In the latter group of compounds superconducting transitions were found for RE given by Y, Nd, Sm, Er, Tm or Lu, but it was later found that in the Er compound (Fertig *et al.* *Phys. Rev. Lett.* **38**, 987; 1977) the superconductivity setting in at 8.7 K disappears with the formation of a state of magnetic long-range order below 0.9 K. The presence of magnetic order was inferred from a pronounced λ type specific heat anomaly and an accompanying susceptibility peak; neutron diffraction experiments have

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since demonstrated (Monckton *et al. Phys. Rev. Lett.* **39**, 1164; 1977) that the order is ferromagnetic.

It is impressive (and to some people surprising) that these properties of $\text{Er Rh}_2\text{B}_4$ can be given a convincing discussion (Jarlborg *et al. Phys. Rev. Lett.* **39**, 1032; 1977) in terms of a calculated electronic structure. That any reasonable form of band structure calculation for a compound containing three types of atom and eighteen atoms per crystal unit cell can now be carried out is a testimony to the enormous progress made in such calculations by the combination of intelligent choices of calculational schemes and large amounts of computing capacity. The superconductivity seems to be associated with a high density of 4d-electron states (per unit energy) at the Rh atom sites, just as in the superconductivity of pure V and Nb (although for other reasons not in pure Rh), while the rather small density of states for 5d electron states on the Er sites, provides a magnetic coupling like that in pure Er between the local 4f moments on these sites. This, while not as strong as that between the larger 4f moments which completely suppresses superconductivity in favour of magnetic ordering in $\text{Gd Rh}_2\text{B}_4$, does finally, below 0.9 K, enable magnetic order to appear.

It seems not unreasonable that a long range magnetic order of ferromagnetic character should be incompatible with superconductivity since there is no sharp separation between two types of conduction band electrons (4d-like on Rh and 5d-like on Er), and the long-wave-length (zero wave-vector) spontaneous parallel spin pairing which is the essence of ferromagnetism cannot coexist with the Fermi surface antiparallel pairing which is the essence of normal superconductivity. The other forms of pairing which parallel the superfluidity of liquid ^3He are, as yet, only gleams in the eyes of theoreticians.

In other groups of compounds, the Chevrel phases $\text{RE Mo}_6\text{S}_8$ and $\text{RE Mo}_6\text{Se}_8$, there has, however, been some evidence for the coexistence of superconductivity and long range magnetic order. Thus, the La Jolla group (see the review by Maple in *Transition Metals*, 655; 1977 (Inst. Physics Conf. Series No. 39, 1977)) have found specific heat anomalies below T_c in $\text{RE Mo}_6\text{S}_8$ for Sm, Gd, Tb, Dy and Er, which they ascribe to the formation of a state of magnetic long-range order, probably antiferromagnetic, within the superconducting phase field. In view of the important theoretical implications of such a coexistence (in formal terms a divergence in the wave-vector dependent susceptibility $\chi(q)$ at some antiferromagnetic wave vector q_0 while

$\chi(0)$ goes to zero) it is of great interest that it has proved possible very recently to show the antiferromagnetism of the Dy compound unambiguously by neutron diffraction techniques.

In a recent paper (Monckton *et al. Phys. Rev. Lett.* **41**, 1133; 1978), research workers from the pioneer Geneva group, from Bell Labs and from Brookhaven have used the Brookhaven neutron scattering facilities to examine a specimen of $\text{Dy Mo}_6\text{S}_8$ down to a temperature of 50 mK, and have shown conclusively that this material (which becomes superconducting at 2.05 K) develops long-range antiferromagnetic order at 0.4 K. Conventional powder diffraction patterns taken above and below this temperature, together with high resolution scans of certain peaks, have permitted the identification of the detailed magnetic structure and yielded estimates of the ordered moments, which show some suppression by crystal field effects from the free Dy^{3+} ion values.

The coexistence of superconductivity and antiferromagnetic long-range order is now proven; a theoretically satisfactory account of such coexistence has yet to be produced. $\square$

A star called M

from S. Jocelyn Bell Burnell

IN THE good old days (that is more than 10 years ago) people believed that neutron stars would only be detectable (if at all) through their steady, thermal optical emission. Calculations of the expected brightness are difficult to perform realistically; heat is generated in bizarre circumstances by friction between charged particles and a neutron superfluid in the interior of the star and there is heat loss by radiation from the surface, but the character of this radiation is drastically modified by the presence of a magnetic field of about 10^{12} gauss. Indications are that the surface temperature should be about 10^6 K and that the most readily detectable neutron stars would, optimistically, be within the grasp of the world's largest telescopes or, more realistically, might be just beyond them.

In the event, however, neutron stars were detected much more readily by radio astronomers (as pulsars) and soon afterwards by X-ray astronomers who concluded that many of the strong

X-ray sources in our Galaxy were powered by a neutron star's gravity. Radio astronomers now know of some 300 pulsars. All pulse regularly, and their periods range from 33 ms to about 4 s. But only two of these three hundred have been detected at optical wavelengths. They are two of the most rapid pulsators—those in the Crab and Vela nebulae—and it was through their pulsed optical emission that the star responsible was ultimately identified in each case.

The Vela pulsar proved difficult to detect at optical wavelengths. At one stage astronomers despaired of detecting pulses and looked for a star whose emission might be accounted for as steady thermal radiation of a hot neutron star. On these grounds Lasker suggested as candidate a star he called M. That star called M turned out to be the pulsar.

Now events have taken another interesting turn bringing us back, almost, to where we started. The group that discovered the optical pulsations from Vela report (Peterson *et al. Nature* **276**, 475; 1978) that some 50% of the optical emission from this star is unpulsed. A series of frames taken during the pulsation cycle of the star shows quite clearly the double-pulsed profile which is its signature, and some residual light during the 'off' part of the pulsar's duty cycle. However, there is too much steady emission for it to be thermal emission from the surface of the star and they are forced to consider other possibilities. Perhaps it is the pulsar emission reflected off a cloud of gas or off the surface of the star; perhaps there are several sources of emission around the star which blur together; perhaps there are very broad pulses which fill the whole cycle.

They find a similar but perhaps less dramatic effect for the Crab pulsar. Here again there were technical difficulties, but this time it was the detection of the weak, steady emission in the face of the short but bright flash from the pulsar. Whereas the Vela pulsar needed 8 hours' observation with the 3.9 m Anglo-Australian Telescope, the Crab pulsar needed only 10 minutes' observation with the telescope stopped down to approximately half aperture! The conclusion is that the Crab too has steady optical emission, and although it's a smaller proportion of the total emission once again it is too copious to be accounted for by a thermal mechanism.

It will be interesting to see whether the latest generation of EUV and soft X-ray astronomy satellites can observe a similar effect. Meanwhile these incredible stars continue to puzzle, and occasionally to surprise. Less surprising will be the inevitable spate of papers which endeavour to explain this latest result. $\square$

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review article

A gene complex controlling segmentation in *Drosophila*

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The bithorax gene complex in Drosophila contains a minimum of eight genes that seem to code for substances controlling levels of thoracic and abdominal development. The state of repression of at least four of these genes is controlled by cis-regulatory elements and a separate locus (Polycomb) seems to code for a repressor of the complex. The wild-type and mutant segmentation patterns are consistent with an antero-posterior gradient in repressor concentration along the embryo and a proximo-distal gradient along the chromosome in the affinities for repressor of each gene's cis-regulatory element.

THE segmentation pattern of the fly provides a model system for studying how genes control development. The phylogeny, although not the ontogeny, of that pattern is reasonably well understood. Flies almost certainly evolved from insects with four wings instead of two and insects are believed to have come from arthropod forms with many legs instead of six. During the evolution of the fly, two major groups of genes must have evolved: 'leg-suppressing' genes which removed legs from abdominal segments of millipede-like ancestors followed by 'halter-promoting' genes which suppressed the second pair of wings of four-winged ancestors. If evolution indeed proceeded in this way, then mutations in the latter group of genes should produce four-winged flies and mutations in the former group, flies with extra legs. In *Drosophila*, not only have both types of mutation been observed, they have been shown to involve a single cluster of pseudoallelic genes known as the bithorax complex (BX-C)¹⁻⁷. During evolution a tandem array of redundant genes presumably diversified by mutation to produce this complex^{1,4}. During development the BX-C genes seem, on the basis of evidence to be summarised in this article, to control much of the diversification of the organism's thoracic and abdominal segments. It is as if during ontogeny the BX-C genes recapitulate their own phylogeny.

Each of the wild-type thoracic and abdominal segments has a unique pattern of differentiated structures (Fig. 1a) which constitutes a morphologically defined state or 'level of development' (L). Each BX-C mutant phenotype can be described in terms of the degree to which one or more segments, or portions thereof, are transformed from one such level to another. As shown in Fig. 1b a striking feature is that the metathoracic (MT) and first three abdominal segments (AB1, AB2 and AB3) can approach or achieve almost all segmental levels of development from a mesothoracic level (LMS), or 'primitive' level¹, to a third abdominal level (LAB3), depending upon the genotype. The wild-type allele of each BX-C gene (Fig. 2) will be assumed to code for a BX-C substance (S) which controls one or more components of an intersegmental transformation (Table 1). The various BX-C substances are presumed to act indirectly by repressing or activating other sets of genes which then directly determine the specific structures and functions that characterise a given segment. On such a model the level of development which a segment achieves depends upon the particular array of BX-C substances elaborated in that segment. When the wild-type and mutant phenotypes are interpreted in accordance with this model, it can be seen from Fig. 1b that the attainment of any level more

Table 1 Summary of the roles of BX-C substances (S) in controlling specific types of body segment transformations and specific structures in one or more segments of the larva

Gene	Substance	Segmental transformation	Larval structures affected	Genotypic comparisons (from Fig. 6)
<i>bx</i> ⁺	S ₁ *	LAMS → LAMT	DLT	<i>a</i> vs <i>b</i>
<i>pbx</i> ⁺	S ₂	LPMS → LPMT		<i>f</i> vs <i>h</i>
<i>Ubx</i> ⁺	S ₀ *	LMS → LMT		<i>g</i> vs <i>h</i>
<i>bx</i> <i>d</i> ⁺	S ₃	LMS → LAB1	DLT†, VP, KO, VSB	<i>b</i> vs <i>c</i>
<i>iab-2</i> ⁺	S ₄	LMS → LAB2		<i>c</i> vs <i>h</i>
<i>iab-3</i> ⁺	S ₅	LAB2 or LMS → LAB3	DLT, KO, VSB	<i>e</i> vs <i>f</i>
<i>iab-5</i> ⁺	S ₇	LAB4 or LMS → LAB5		
<i>iab-8</i> ⁺	S _x	LAB7 or LMS → LAB8		<i>a</i> vs <i>d</i>
			DLT, PSP, CP, VP‡, KO‡	

* Substance, S₁, was originally postulated to be coded for by either *bx*⁺ or *Ubx*⁺ (ref. 4); in this article S₁ is assigned to *bx*⁺ and S₀ to *Ubx*⁺.

† Comparison of genotype *e* and *f* indicates that *bx**d*⁺ and/or *iab-2*⁺ may be responsible for the continuity of dorsal tracheal trunk in AB1.

‡ Suppression of VP and KO may result from the presence of *iab-8*⁺ and/or one or more *iab* genes located between *iab-3* and *iab-8*.

a

DSC
Mro
ASP
VP
KO
VSB
DLT
PSP
HEAD
PRO
MS
MT
AB₁
AB₂
AB₃
AB₄
AB₅
AB₆
AB₇
AB₈
C1
C2
W
H
SS
WO
ST
MSI
MTL
LARVA ADULT ♀
(VENTRAL ASPECTS)

b

Ub^{x+} bxd⁺ iab-2⁺ iab-3⁺

GENOTYPE
LEVEL OF DEVELOPMENT

HEAD
PRO
WILD TYPE
MS
Ubx⁺ Cbx / +
LMT

Ubx Ubx⁺ WILD TYPE LMS MT

iab-2⁺ Hab / + LAB2

iab-3⁺ Hab / Uab³ LAB3

Ubx Ubx⁺ bxd bxd⁺ WILD TYPE LMS LMT

iab-2⁺ Hab / + LAB2

iab-3⁺ Uab³ / + LAB3

Ubx Ubx⁺ Dfp9 Dfp9⁺ LMS LMT

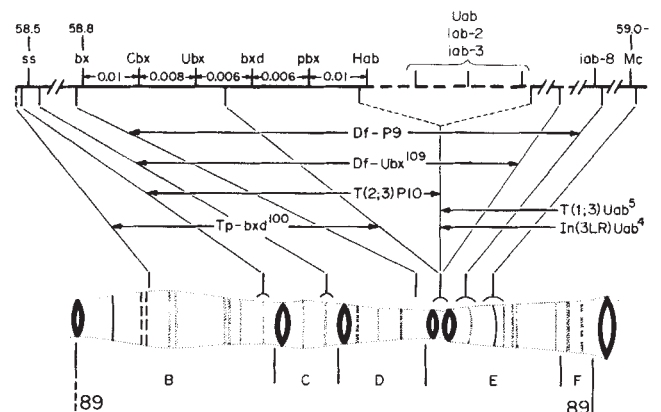
iab-2⁺ iab-3⁺ WILD TYPE LAB2 LAB3

RELATIVE AMOUNT OF HYPOTHETICAL GENE PRODUCT (S)

- WILD TYPE
- ◐ SLIGHTLY REDUCED
- ◑ STRONGLY REDUCED
- NONE

Fig. 2 Correlation of the linkage and salivary gland chromosome maps of BX-C and immediately surrounding regions in the right arm of the third chromosome. The solid line portion of the linkage map is based on half-tetrad⁶, as well as on conventional, linkage analysis. The dashed line portion is based on unpublished cytogenetic studies of the designated rearrangements, the relative order of *Hab*, *Uab*, *iab-2* and *iab-3* with respect to each other being uncertain. The complex spans approximately 0.05 to 0.1 centimorgan and lies within the region of two heavy doublet structures and possibly an adjoining faint band of section 89E. Not shown is a spontaneous dominant mutant, Miscadestral pigmentation (*Mcp*), recently found by M. Crosby, which maps between *Hab* and *Microcephalus* (*Mc*)¹⁵. *Df-P9* in its interactions with BX-C mutants and rearrangements acts as if it is deleted for all of the BX-C genes. *Df-P9/+* has reduced male pigmentation in AB5 and AB6, is sterile, and has deformed genitalia in both sexes, which on the basis of other interactions and of larval studies (Fig. 1) weakened dominance of genes to the right of *iab-3*. *Mc* is apparent to *Df-P9* but does not otherwise modify the *Df-P9/+* phenotype. section 29C of chromosome 2; the breakage point in 89E is likely polar position effects that weaken the dominance of *iab-2*⁺ and chromosome 3L; the breakage point lies between *Ubx* and *bxd* and as far as can be detected, of genes to the right of *pbx*. *T(1;3)Uab* chromosome; a synthetic deletion analysis indicates that the *Uab*⁵ breakage point in 89E lies to the left of *Uab*⁵ and falls either to the left of 80C and in or near 85A or 3R. Analysis of X-ray induced breaks to the left or right of *iab-2* and is associated with a weakened dominance of *Hab* was originally designated as the F locus of the complex⁷; and *Uab* mutants found, *Uab* and *Uab*², although cytologically apparent complex and are accompanied by position effects or point mutations *Uab*¹ much more closely than that of *Uab*⁵. Cellular determi-

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AB5 and AB6, is sterile, and has deformed genitalia in both sexes, which on the basis of other interactions and of larval studies (Fig. 6d) behaves as a deficiency extending from *bx* to *iab-3*, associated with a weakened dominance of genes to the right of *iab-3*. *Mc* is apparently a very small tandem duplication which partially restores fertility when *trans* to *Df-P9* but does not otherwise modify the *Df-P9/+* phenotype. *T(2;3)P10* is an insertional translocation of the designated region of 89 to section 29C of chromosome 2; the breakage point in 89E is likely on genetic grounds to lie between *pbx* and *iab-2* and to be accompanied by polar position effects that weaken the dominance of *iab-2⁺* and *iab-3⁺*. *TP(3)bx^d100* is a transposition of the designated 89 region to 66C of chromosome 3L; the breakage point lies between *Ubx* and *bxd* and is accompanied by a weakened dominance of *bdx⁺* and *pbx⁺* (ref. 1) but not, as far as can be detected, of genes to the right of *pbx*. *T(1;3)Uab⁵* is a reciprocal translocation with a breakage point in section 1E of the X chromosome; a synthetic deletion analysis indicates that the *Uab⁵* phenotype is associated with the distal portion of the translocation; that is, the breakage point in 89E lies to the left of *Uab⁵* and falls either to the left or right of *iab-2⁺*. *In(3LR)Uab⁴* is a complex inversion with additional breaks in 80C and in or near 85A or 3R. Analysis of X-ray induced revertants of *Uab⁴* indicates that the breakage point in 89E probably lies just to the left or right of *iab-2* and is associated with a weakened dominance of *iab-3⁺* (designated in Fig. 1 *iab-3^{Uab⁴}*) and possibly of *iab-2⁺*, as well. *Hab* was originally designated as the F locus of the complex⁷; and has also appeared in the literature as Contrabithoraxoid (*Cbx^d*). The first two *Uab* mutants found, *Uab* and *Uab²*, although cytologically apparently normal, are associated with a reduction in crossing over within the complex and are accompanied by position effects or point mutations at other loci within BX-C. Their dominant phenotypes resemble that of *Uab⁴* much more closely than that of *Uab⁵*. Cellular determination of *Uab* has been extensively studied by Kiger and Davis^{20,21}.

advanced than LMS is a stepwise process in which each step requires the presence of a specific BX-C substance. In keeping with the model, all of the genotypes involving recessive loss of function lie to the left or LMS side of the wild-type segments while those involving dominant gain of function lie to the right or LAB3 side of those segments.

Segment MT transforms towards LMS even in larval stages of homozygotes¹ and hemizygotes for Ultrabithorax (*Ubx*). The most prominent feature is the occurrence of an extra set of spiracles on MT (and also AB1) (Fig. 3a) compared to the usual single set on MS. Although such animals die in late larval or early pupal stages, adult *Ubx/Ubx* cuticular tissue in MT has been shown by somatic mosaic analysis to transform autonomously towards LMS^{4,8}. A substance, S_0 , effecting LMS $\rightarrow$ LMT, will be assumed to be the *Ubx*⁺ product. The inability of MT to achieve LMT in *Ubx* hemizygotes or homozygotes is then consistent with the expected reduction in amount of S_0 in that segment. The adult MT cuticle, but not the larval tracheal system, strongly transforms toward LMS in a double mutant combination involving bithorax-3 (*bx*³) and postbithorax (*pbx*), notably producing a four-winged fly^{5,8}. Substances S_1 and S_2 are assumed to be the products of *bx*⁺ and *pbx*⁺, respectively (Table 1), and to be involved in effecting LMS $\rightarrow$ LMT; however, for the sake of simplicity, they are omitted from Fig. 1b. The wild-type phenotype, on these assumptions, corresponds to that expected if *bx*⁺, *Ubx*⁺ and *pbx*⁺ are repressed in MS and derepressed in MT.

Segment MS takes on MT characteristics in flies heterozygous or homozygous for Contrabithorax (*Cbx*), the most conspicuous effect being a partial conversion of wings into halteres^{5,9}. *Cbx* is known to regulate *Ubx*⁺ in a cis-dominant fashion; that is, MS transforms towards LMT in *Cbx* $+/+$ *Ubx* but is virtually wild type in *Cbx Ubx* $+/+$ ^{2,5}. *Cbx* presumably damages a regulatory element adjacent to *Ubx*⁺ causing S_0 to be made constitutively in MS but in a relatively reduced amount compared with that made in MT when *Ubx*⁺ is in the wild-type configuration.

Segment AB1 transforms primarily towards LMT in hemizygotes for bithoraxoid (*bxd*), producing flies with one or two MT-like legs on AB1, and on rare occasions a tiny haltere-wing as well⁵. This phenotype has been accounted for by postulating that *bxd*⁺ codes for a substance, S_3 , effecting LMS $\rightarrow$ LAB1⁴. Evidence to be represented later suggests that *Ubx*⁺ is derepressed not only in MT but also in AB1 and segments beyond. Hence, in the *bxd* hemizygote, there is presumably enough S_0 in AB1 to transform that segment towards LMT. That AB1 remains at LMS in the *Ubx* hemizygote (Fig. 3a) is expected since *Ubx* is known to exert a strong polar effect on *bxd*⁺ (ref. 1); that is, neither S_0 nor S_3 is expected to be made in sufficient amounts to transform AB1 appreciably towards LMT or LAB1. In wild type, S_0 as well as S_3 is presumably being produced in AB1; although S_3 function may override that of S_0 , a more attractive possibility is that LAB1 actually corresponds to a mosaic of structures and functions controlled jointly by S_0 and S_3 .

Segments MT and AB1 approach LAB2 in heterozygotes for a second type of dominant regulatory mutant, Hyperabdominal (*Hab*), producing flies with missing halteres and/or MT legs and with an AB2-type of sternite pattern^{10,11} on MT and AB1 (Fig. 4b). These effects are variably expressed within a fly even though the penetrance of *Hab* $+/+$ exceeds 99% in certain backgrounds.

Cis-trans studies involving all possible double mutant combinations between *Hab* and *bx*³, *Ubx*, *bxd* and *pbx* indicate that *Hab* does not derepress the wild-type alleles of any of these genes. *Hab* will be assumed to cause partial derepression of a wild-type infra-abdominal-2 (*iab-2*⁺) gene, whose coding product, S_4 , effects LMS $\rightarrow$ LAB2. On this assumption, the wild-type phenotype is that expected if *iab-2*⁺ is repressed in MT and AB1 and derepressed in AB2. Similarly, the *Hab* $+/+$ phenotype is that expected if *iab-2*⁺ is partially derepressed in MT and AB1 and fully derepressed in AB2.

The failure of MS to be modified in *Hab* $+/+$ requires explanation. The *Hab* mutation presumably damages a regulatory element adjacent to *iab-2*⁺ in such a way as to reduce its affinity for a repressor. If there is an antero-posterior concentration gradient in that repressor, then conceivably the threshold concentration for keeping *iab-2*⁺ repressed in the *Hab* chromosome is exceeded in MS but not in MT and segments beyond MT.

Segments AB1 and AB2 partially transform toward LAB3 in adults heterozygous for Ultra-abdominal-5, *Uab*⁵, a third type of dominant constitutive mutant, inseparable from an X-3 translocation (Fig. 2). The transformation is most easily seen in the ventral regions of AB1 and AB2 which develop sternite patterns approaching those found in AB3 (Fig. 4c). The effect on AB2 can be accounted for if *Uab*⁵ derepresses a gene, *iab-3*⁺, which codes for a substance, S_5 , effecting LAB2 $\rightarrow$ LAB3. The wild-type pattern then corresponds to that expected if *iab-3*⁺ is repressed in AB1 and AB2, but derepressed in AB3. The penetrance of *Uab*⁵ $+/+$ is complete but the transformation of AB1 towards LAB3 is variable with that segment often progressing only towards LAB2. If, as certain larval findings discussed below suggest, *iab-2*⁺ is normally weakly derepressed in AB1, then the presence of S_4 in AB1 even in subthreshold amounts may facilitate the transformation to LAB3 in *Uab*⁵ $+/+$. Another possibility is that the *Uab*⁵ rearrangement in some way partially derepresses *iab-2*⁺ as well as *iab-3*⁺; however, although MT transforms to LAB2 in *Hab* $+/+$, MT is not modified in *Uab*⁵ $+/+$.

Segment MT is able to approach LAB3, along with AB1 and AB2, in *Hab/Uab*⁵ animals (Fig. 4d). Transformation to LAB3 in MT thus seems to require two sequential steps: LMS $\rightarrow$ LAB2 $\rightarrow$ LAB3, the former controlled by S_4 and the latter by S_5 . The observed transformation of all three segments towards LAB3 is readily accounted for, since both S_4 and S_5 are expected to be produced in MT, AB1 and AB2 of *Hab/Uab*⁵.

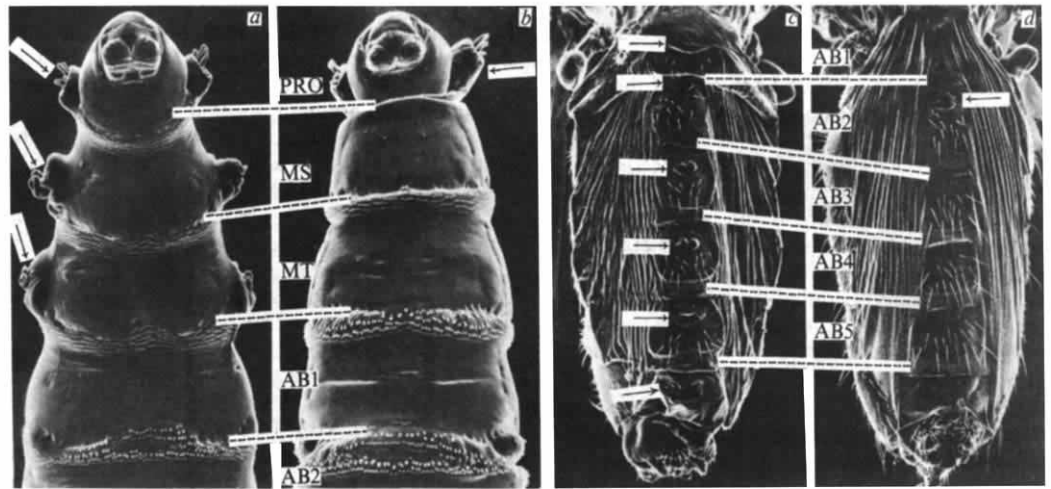
Additional evidence for an *iab-3*⁺ gene comes from an analysis of another rearrangement-associated *Uab* mutant, *Uab*⁴. When heterozygous, *Uab*⁴ shows a dominant gain of function in which AB1 partially transforms towards LAB2. When hemizygous, or opposite *T(2; 3)P10* (Fig. 2), *Uab*⁴ exhibits a new type of recessive loss of function; namely, the sternites of not only AB1 but AB3 to AB6, inclusive, transform towards LAB2 (Fig. 3c). These latter effects are attributed to a reduction in the amount of S_5 which would be expected if the *Uab*⁴ and *T(2; 3)P10* rearrangements are accompanied by position effects on *iab-3*⁺.

Gonads, normally located in AB5, are absent in *Uab*⁴ hemizygotes, suggesting that BX-C genes control mesodermal as well as ectodermal tissues. Gonadal examination of *Uab*⁴ genotypes was prompted by the report of gonadal defects in homozygotes for certain *Uab*-like mutants of the homologous 'E-locus' complex of *Bombyx* (see review by Tazima¹²).

Segment AB4 transforms strongly towards LAB5, both in pigmentation and bristle patterns in heterozygotes and homozygotes for Mescadestral pigmentation (*Mcp*), a dominant mutant recently discovered by M. Crosby (unpublished). Analysis of X-ray induced revertants indicates that *Mcp* probably derepresses a gene, *iab-5*⁺, which codes for a substance, S_7 , effecting either LAB4 $\rightarrow$ LAB5 or LMS $\rightarrow$ LAB5.

A new dimension has been added to the analysis of BX-C with the discovery that a homozygous deficiency for the entire complex, *Df-P9* (Fig. 2) survives to the late embryonic or early first instar stage and exhibits a transformation of MT and all eight AB segments towards LMS. Pairs of Keilin's organs¹³ (KO), ventral pits (VP) and thoracic-type ventral setal bands (VSB) appear on those segments (Fig. 5a and 5b) and in place of a continuous dorsal longitudinal tracheal trunk (DLT) each segment bears a section of DLT terminating presumably in an incipient anterior spiracle (ASP)¹⁴ (Fig. 5c, vs. d); finally, the ventral nerve cord tends to retain its primitive embryonic pattern in that it extends to segment AB6 or AB7 instead of foreshortening to segment AB3 or AB4 as in wild type. Tiny

Fig. 3 Mutant phenotypes resulting from recessive loss of function compared with wild type. See Fig. 1(a) for legend. *a*, Late third instar larva hemizygous for *Ubx* (*Ubx/Df-P9*). Note extra pairs of anterior spiracles (ASP) (arrows) on MT and AB1, protruding into MS and MT, and thoracic type of ventral setal bands (VSB) on AB1 compared with abdominal type on AB2. *b*, Corresponding stage of homozygous wild-type (Canton-S) larva. Note single pair of ASP (arrow) which arises in MS but protrudes into the region of the prothorax (PRO). Note setae of VSB are coarse in AB1 and AB2, fine in MS, and intermediate in MT. (Specimens *a* and *b* were fixed in hot water and photographed with SEM, X40. *c*, Ventral aspect of male abdomen of genotype *In(3LR)Ubx⁴/T(2; 3)P10* (see Fig. 2). Note presence of WO (arrows) and sternital bristle pattern of AB2 type on segments AB1 through AB5. Gonads are rudimentary in specimens of this genotype or lacking entirely in *Ubx⁴/Df-P9*. *d*, Corresponding region of adult wild-type (Canton-S) male. Note that AB1 lacks a sternite and that only AB2 (arrow) has WO. (Specimens *c* and *d* were unfixed and photographed with SEM, X50.)



chitinised plates (CP), resembling rudiments of mandibular hooks (MH), occur in AB8 and imply a head-like rather than LMS transformation of one or more of the embryonic segments that normally fuse to form AB8.

In order to delineate regional differences in morphological functions within BX-C, hemizygotes for deficiencies of portions of BX-C have been constructed. Figure 6 displays stylised phenotypes associated with genotypes hemizygous for five such deficiencies (*b-f*), for *Df-P9* (*a*), for *bx^d* (*g*) and for wild type (*h*).

A direct demonstration that *Ubx⁺* effects LMS → LMT can be seen by comparing genotypes *a* and *b*, which lack all BX-C genes except that *b* retains one dose of *Ubx⁺* (and *bx⁺*). All nine segments from MT to AB8 remain at LMS in *a* and attain LMT in *b*; that is, in *b* they acquire a pattern of attributes unique to the

larval MT (namely, a continuous DLT, VP, KO and thoracic-type VSB). Furthermore *Ubx⁺* behaves as if derepressed not only in MT and AB1, but in the remaining abdominal segments as well. That many other BX-C genes share with *Ubx⁺* the ability to establish continuity of DLT is evidenced by the presence over several segments of continuous DLT in larval genotypes, *d*, *e* and *f*, which lack *Ubx⁺* but have one or more other BX-C genes still present.

All abdominal segments approach, but do not quite achieve, LAB1 in animals of genotype *c*, which is a hemizygote for *bx^d* (and *pbx⁺*) as well as *Ubx⁺*. Such animals are seen to have most of the characteristics of wild-type AB1 (absence of ASP and VP and presence of DLT and VSB, but incomplete suppression of KO). Failure of *bx^d* to effect a perfect transformation to LAB1 is not understood. One possibility is that *iab-2⁺* and *bx^d* are

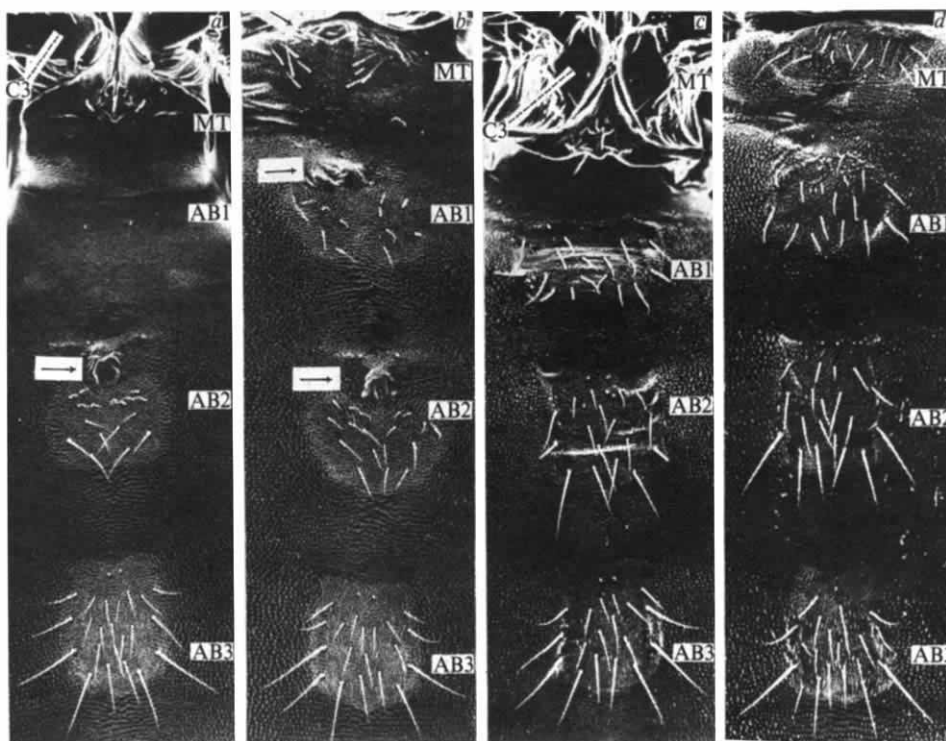


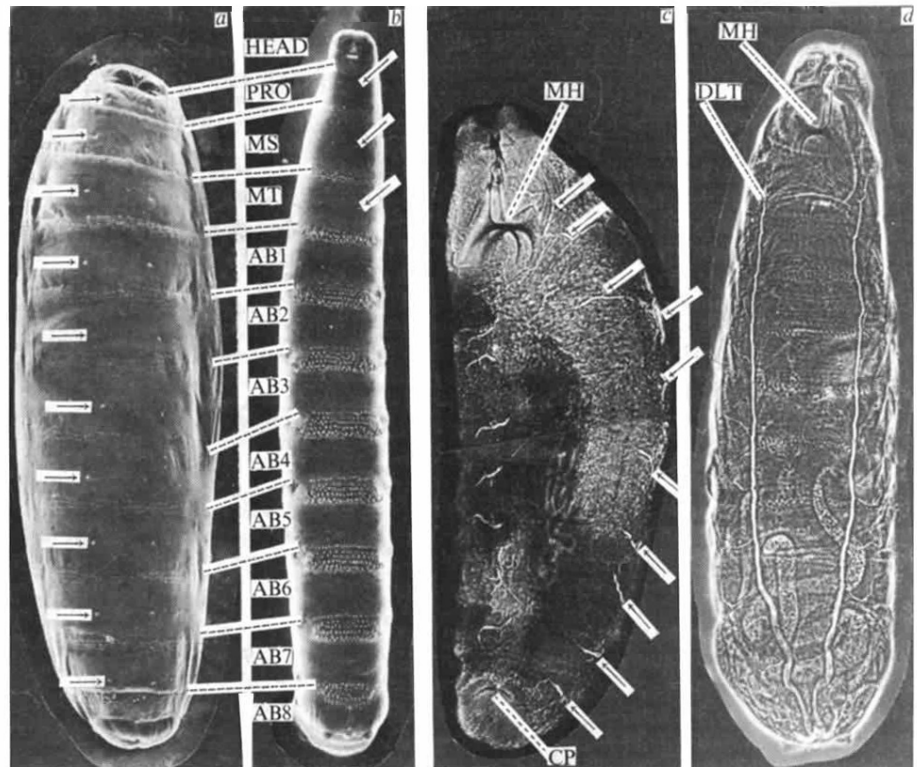
Fig. 4 Mutant phenotypes resulting from dominant gain of function compared with wild type. Mid-ventral cuticular regions of adults female with MT, AB1, AB2 and AB3 approximately parallel to one another. (All specimens unfixed and photographed with SEM.) *a*, Wild type. A portion of coxa-3 (C3) is visible on MT; AB1 lacks a sternite; AB2 has WO (arrow) and a reduced (compared to AB3) sternital bristle pattern, X80. *b*, *Hab/+*. The specimen had four legs and no halteres. WO (arrows) and bristle pattern on MT and AB1 approach those of AB2, X80. *c*, *Ubx³/+*. C3 resembles that of wild type. AB1 and AB2 sternites lack WO and approach in bristle pattern that of AB3, X90. *d*, *Hab/Ubx⁵*. The specimen had four legs and no halteres. Sternites on MT, AB1 and AB2 have little or no trace of WO and approach in their bristle patterns that of AB3, X80.

both involved in the suppression of KO and that *iab-2*⁺ is weakly derepressed in AB1 (depicted in Fig. 6*h* by indicating a strongly reduced amount of S₄ in that segment of wild type). Complete suppression of KO can occur in genotypes which lack full *bxd*⁺ function; namely, *f* and *g*. In such cases, it seems likely that *iab-2*⁺ and possibly *iab-3*⁺ are able to bring about suppression of KO when fully derepressed.

Suppression of VP seems to be exclusively controlled by *bxd*⁺, these organs being absent from abdominal segments only when *bxd*⁺ is present, as in genotypes *c* and *h*. It is therefore directly evident from *h* that *bxd*⁺ is normally derepressed not only in AB1, but in all segments posterior to AB1.

would derepress or activate BX-C genes in all segments of the body. The properties of Polycomb (*Pc*), a mutant found by P. H. Lewis¹⁵, and of a more extreme allele, *Pc*³, suggest that *Pc*⁺ (locus 3-47.1)¹⁶ is such a gene. Thus, hemizygotes and homozygotes for *Pc* or *Pc*³ have recently been found to have their thoracic and first seven abdominal segments partially transformed towards LAB8. Such animals, which die as late embryos, also have reduced head structures, including poorly developed and incompletely sclerotised MH. As the doses of BX-C are increased from two to three to four, the transformation towards LAB8 becomes increasingly more extreme with many of the abdominal segments containing rudimentary posterior-type

Fig. 5 Comparison of cuticular and tracheal patterns in homozygotes for a deletion of BX-C (*a* and *c*) compared with those of wild type (*b* and *d*). See Fig. 1 for legend. *a*, *Df-P9* homozygote. Animals die within the egg or after partly emerging from egg membranes. Note thoracic-like VSB, KO (arrows) on segments AB1 through AB7, as well as on thoracic segments; AB8 has a reduced VSB. KO not visible in AB8 of specimen shown but are visible in whole mounts examined under phase contrast, $\times 160$. *b*, Wild-type first instar larva. Note more prominent VSB on abdominal vs. thoracic segments, with VSB on MT somewhat intermediate between those on MS and AB1. KO (arrows) are restricted to thoracic segments as are also VPs, which are not resolved at the magnification shown, $\times 60$. (Specimens *a* and *b* were fixed in hot water and photographed with SEM.) *c*, Whole mount of mature embryo of *Df-P9* homozygote. Note separate tracheal sections (arrows) of DLT occur in each segment from MS through AB8, and tiny chitinized plates (CP) in AB8. *d*, Whole mount of wild-type first instar larva. Note continuous DLT. (Specimens *c* and *d* mounted in Zeiss W15 medium and photographed under phase contrast, $\times 120$.)



The larval effects of *iab-2*⁺ can be indirectly inferred by comparing tracheal and cuticular patterns in AB2 of genotypes *d*, *e* and *f* (Fig. 6), which involve loss, weakened dominance and dominance, respectively, of *iab-2*⁺. Continuity of DLT is seen in both *e* and *f*, suppression of KO occurs only in *f*, and VSB become progressively more abdomen-like in proceeding from *d* to *e* to *f*. These effects are not necessarily solely due to the *iab-2*⁺ gene since it is possible that *iab-3*⁺ is weakly derepressed in AB2 of the larva. Unfortunately, definitive genotypes are not available that would resolve *iab-2*⁺ and *iab-3*⁺ larval functions.

A comparison of larvae of genotypes *a* and *d* (Fig. 6) shows that the latter retain the wild-type cuticular pattern in AB8 (except for thoracic-type VSB) and have DLT continuity between AB7 (or AB6) and AB8. These effects are most simply accounted for by assuming that in contrast to *Df-P9*, *Df-Ubx*¹⁰⁹ still retains an *iab-8*⁺ gene, whose product, S_x, effects LAB7 → LAB8 or LMS → LAB8. The ability of genotype *d* to restore a tracheal trunk in the region from AB6 to AB8 suggests that *Ubx*¹⁰⁹ retains additional *iab* loci to the right of *iab-3*, besides *iab-8*.

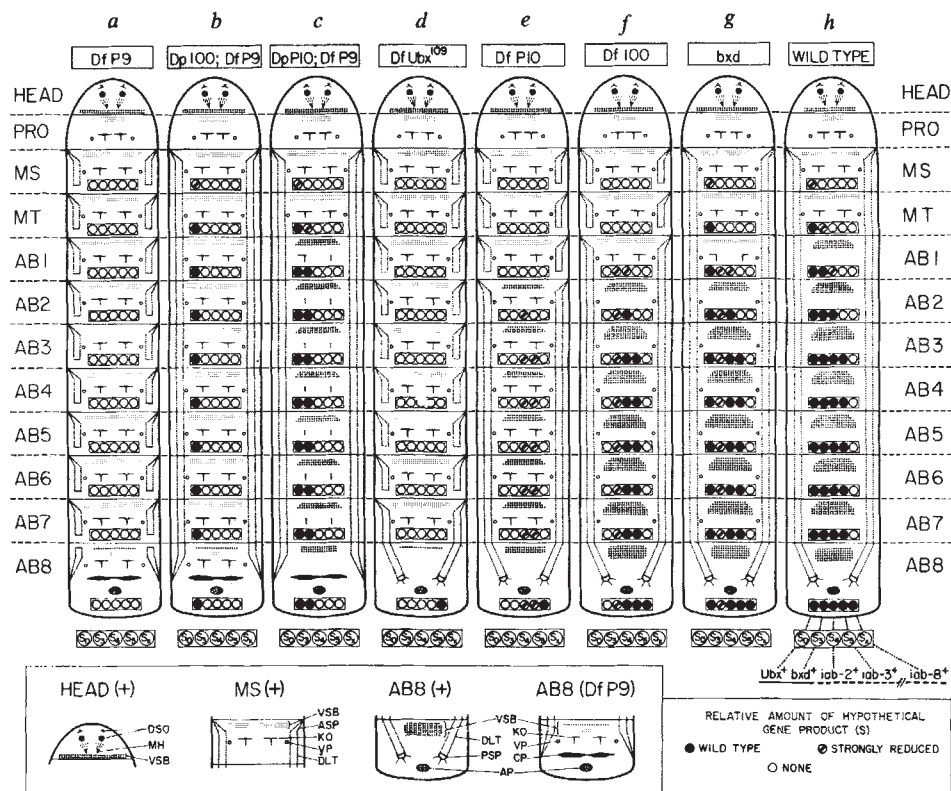
The above findings point to an antero-posterior gradient within the organism in BX-C gene action. A regulatory gene(s) might therefore be expected to exist which, when activated,

spiracles. The LAB8 transformation is not observed unless at least one dose of BX-C is present; that is, *Pc*³ *Df-P9* homozygotes closely resemble *Df-P9* homozygotes (Fig. 5) except that the former have reduced head structures and incompletely sclerotised CP in AB8 (suggesting that the latter structures are indeed rudiments of MH). A dependence of BX-C phenotypic effects on BX-C dosage is seen also in *Pc*³ heterozygotes: for example, *Cbx*-like effects occur in *Pc*³/+ animals (with two doses of BX-C) and become increasingly more extreme with three and four doses of BX-C. Gene dosage studies of R. Denell (personal communication) indicate that *Pc* represents the inactivated state of the gene. Therefore, *Pc*⁺ in all likelihood is coding for a repressor of BX-C.

Regulatory rules

The BX-C genes are assumed to control the organism's thoracic and abdominal segmentation pattern by producing substances which in turn regulate other genes that actually determine segmental structure and function. Regulation of the BX-C genes, themselves, seems to be governed by the following rules: (1) state of repression or derepression of a given gene is controlled (in at least four instances) by a *cis*-regulatory element; (2) the genes tend to be individually, rather than coor-

Fig. 6 Stylised phenotypes detectable in first instar larvae or mature embryos. See Fig. 1(a) for legend, Fig. 2 for description of rearrangements and Table 1 for roles of postulated BX-C substances (S). All drawings based on whole mounts of animals examined with phase contrast microscopy. Mounting fluid: 9 parts lactic acid to 1 part 95% ethanol for revealing cuticular structures; and Zeiss W15 for tracheal structures. *a*, *Df-P9* homozygote (see also Fig. 5a and c). *b*, *Df-P9* homozygote containing one dose of *Dp-100*, abbreviated symbol for the duplication derived from *Tp(3)bx^d100* (Fig. 2). Note identity to *a* except for presence of DLT. *c*, *Df-P9* homozygote containing one dose of *Dp-P10*, abbreviated symbol for the duplication derived from *T(2;3)P10* (Fig. 2). Note suppression of VP and partial suppression of KO in segments AB1 to AB7. Although VP and KO appear to be absent in AB8, they may be obscured by CP and other structures in that segment. *d*, *Df-Ubx¹⁰⁹/Df-P9*. Note DLT between segments AB7 and AB8, and presence of PSP in AB8; and absence of CP in AB8; occasionally DLT also forms between AB6 and AB7. *e*, *Df-P10/Df-P9*. *Df-P10* is an abbreviated symbol for the deficiency derived from *T(2;3)P10* (Fig. 2). DLT restored in AB2 through AB8. The pair of separate sections of that trunk in AB1 frequently lie in AB2, parallel to DLT in that segment. Note partial restoration of abdominal-type VSB, weakly developed in AB2 and moderately developed in AB3 through AB8. *f*, *Df-100/Df-P9*. *Df-100* is an abbreviated symbol for the deficiency derived from *Tp(3)bx^d100* and has moderately weakened dominance of *bx^d+* as a position effect of the 89E breakage point. Note retention of DLT and failure of suppression of VP in AB1 through AB7 (with VP status in AB8 uncertain) and full restoration of abdominal type of VSB in all segments from AB2 through AB8. *g*, *bx^d/Df-P9*. Although not deleted for any known genes of BX-C this genotype is depicted since it has effects on several larval (as well as adult) cuticular structures. Note failure of suppression of VP on AB1 through AB7; thoracic-type VSB on AB1; and only partial suppression of KO on AB1. *h*, Wild-type homozygote or hemizygote (the latter having possibly somewhat narrower VSB on AB segments than the former) (see Fig. 5b and d). All individuals of genotype *a* through *f* die in the late embryonic or early first instar stage. ASP are lacking in first instar larvae according to Bodenstein¹⁴. Separate sections of DLT send out a branch to the cuticle but no anterior spiracles as such have been resolved. A number of larval phenotypic expressions are not readily explained unless it is assumed that there is weak derepression of *Ubx⁺* (to establish continuity of DLT between MS and MT), *bx^d+* (to make very slightly larger VSB teeth in MT than MS) and *iab-2⁺* (to suppress KO in AB1) in the segment anterior to that in which each is fully derepressed. Whether *iab-3⁺* or *iab-8⁺* are also similarly weakly derepressed is uncertain.



dinately, derepressed; (3) BX-C is under negative control maintained by a major regulatory gene, *Pc⁺* (along with perhaps other regulatory genes^{7,17}); (4) a gene derepressed in one segment is derepressed in all segments posterior thereto; (5) the more posterior the segment (starting with MT) the greater the number of BX-C genes that are in the derepressed state; and (6) the more proximal the locus of a gene in the complex the more likely it is that that gene is in a derepressed state. Rules (5) and (6) suggest that two types of gradient are involved in BX-C regulation: an antero-posterior gradient within the organism in repressor concentration and a proximo-distal gradient along the chromosome in relative affinity for repressor of *cis*-regulatory elements. Presumably in MT repressor concentration is so high that the only genes derepressed are those whose *cis*-regulatory elements have relatively low repressor affinities, while in AB8 repressor concentration is so low that all of the genes of the complex escape repression.

During ontogeny the above rules presumably result in each segment having a specific array of BX-C substances, at the right time, at the right place. In AB8, for example, the rules predict a minimum of eight such substances, the number of BX-C genes being very likely to exceed the eight identified thus far. Whether such a multiplicity of substances act in a hierarchical or a compartmentalised manner^{18,19} to establish the final pattern or

level of development of a segment may well be accessible to analysis at the cellular level.

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articles

A new class of radio star

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Radio observations of point sources are presented which may be the stellar remains of supernovae. In particular 1909+048 is described, a source which has also been detected in the optical and X-ray ranges.

THE stellar remnants of the Crab and Vela supernovae are detectable over a wide frequency range (radio through optical to X rays) as pulsars showing the short period (<1 s), presumably caused by fast rotation, in all observations. Although it has commonly been assumed that the stellar remnants of all supernovae form pulsars, there have been indications that stellar remnants may sometimes be detectable as non-pulsing radio sources. In particular: (1) Clark *et al.*¹ questioned whether Cir X-1 (with its associated non-pulsing radio source 1516-569) might be the stellar remnant of the supernova which formed the nearby radio shell SNR (supernova remnant) G321.9-0.3; (2) the point radio source 2048+312 (CL4), in the direction of the Cygnus Loop, may be associated² with the SNR; (3) an unusual

point source 0125+628 at the centre of the SNR G127.1+0.5 may be³ the stellar remnant of this supernova. This source has a flat spectrum⁴ between 2.7 and 15 GHz, while its angular size is <1 m arc s and may be much less⁵.

We have recently investigated the point radio source 1909+048 in W50 and have concluded that it certainly represents a distinct new type of radio source. We discuss here this object and some other possible examples for which we have preliminary measurements.

1909+048—the stellar remnant of the SNR W50?

The extended radio source W50 (G39.7-2.0) was recognised as an SNR by Holden and Caswell⁶. More recent data^{7,8} confirm that it has a shell structure and reveal a point source near its centre; a small-diameter X-ray source (W50X) has also been reported near this direction^{9,10}. We have used the Cambridge 5-km radio telescope at 2.7 and 15 GHz to study this point

Table 1 Candidate positions and their possible optical counterparts

Source	Name	Radio position			Mean date of radio position	Possible optical counterpart			Notes
		RA(1950.0 coordinates) dec				RA(1950.0 coordinates) dec			
		h min	s	° ' "		h min	s	° ' "	
0125+628		01 25 08.366 ±0.011		62 50 59.02 ±0.03	1977 May	01 25 08.4		62 50 59.1	Optical position from D. Wills (personal communication)*
0503+466		05 03 40.935 ±0.005		46 41 46.56 ±0.03	1977 April	—		—	No optical candidate visible on Palomar Sky Survey Prints.
1347−617		13 47 04.1 ±0.3		−61 45 54.4 ±2	1977 July	—		—	Position from Fleurs Synthesis Telescope ¹⁶
1516−569	Cir X-1	15 16 48.45 ±0.2		−56 59 11 ±2		15 16 48.51 ±0.06		−56 59 12.7 ±0.5	Positions ¹⁷
1849+005		18 49 13.597 ±0.012		00 31 54 ±2	1977 October	Crowded field			Declination from Fleurs Synthesis Telescope ¹⁶
1909+048	W50X	19 09 21.273 ±0.005		04 53 53.1 ±0.5	1977 March	19 09 21.32 ±0.05		04 53 53.8 ±0.4	See text
1910+052		19 10 26.315 ±0.003		05 12 51.9 ±0.3	1977 May	Blank field			Nothing visible on POSS prints within 20 arc s
2013+370		20 13 37.009 ±0.007		37 01 44.51 ±0.06	1977 March	Crowded field			
2048+312	CL4	20 48 47.380 ±0.002		31 16 10.95 ±0.04	1974−75	See ref. 18*			Radio position ²

* See note added in proof.

source, and have found that the angular size is extremely small, the mean spectrum is relatively flat, and that the flux density varies dramatically at high frequencies (Fig. 1). Since this paper was completed, Seaquist *et al.*¹¹ and Feldman *et al.*¹² have also reported observations of the radio variability, and have noted that the optical object is No. 433 in the list of H α -emission objects by Stephenson and Sanduleak¹³. An accurate position has also been measured (Table 1). The unusual nature of the radio/X-ray source and its position near the centre of the SNR shell strongly suggest that it is the stellar remnant of the supernova. A stellar object is indeed visible on the Palomar Observatory Sky Survey prints which coincides with the radio source to within the limits of error. The optical field is a crowded one, but the star in question is the brightest in the vicinity of the radio source. The estimated magnitudes are $m = 12.4 \pm 0.5$ on the E print and $m = 16.7 \pm 0.5$ on the O print. Clearly, it will be important to obtain an optical spectrum of this star¹⁴, and to investigate the X-ray properties in greater detail.

A comparison of the distances of the point source and the shell SNR is obviously desirable. In the case of the point source, an HI absorption measurement is needed, but for the SNR we have estimated the distance from the Σ -D relation of Caswell and Lerche¹⁵. In reaching this estimate we first reassessed the radio data and adopted a spectral index $\alpha = 0.45$ (where $S \propto \nu^{-\alpha}$), $S(408 \text{ MHz}) = 65 \text{ Jy}$ and elliptical major and minor axes of 81 and 45 arc min respectively. The corresponding surface brightness at 408 MHz, $\Sigma = 2.72 \times 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1}$, yields a distance of 3.3 kpc.

There is also a second point source (1910+052) within the shell of W50 which seems to be of the same type. It is included in Tables 1 and 2, and is discussed later.

Properties of the new class of source

Tables 1 and 2 list the candidates already mentioned, together with some further possibilities. Table 1 gives positions, as measured with the 5-km telescope except in the cases of 1347-617 and 1516-569, and also positions of optical objects which

may be associated. In the case of 1909+048, the optical position has been newly measured from the Palomar Observatory Sky Survey prints, with respect to stars from the Smithsonian Astrophysical Observatory catalogue. Table 2 lists the flux densities at 1.4 GHz, angular diameters (from the 5-km observations if not otherwise noted), spectral indices, variability (see Fig. 1), estimated distances of the SNRs (derived from the Σ -D relationship of Caswell and Lerche¹⁵, except for G74.9+1.2), and associations with SNRs.

From a consideration of the four candidates, 0125+628, 1516-569, 1909+048 and 2048+312, we suggest the following characteristic properties of this class of source: (1) small diameter; (2) variability at high frequencies; (3) flat spectrum at high frequencies; (4) association with an SNR. In general a position near the centre is expected unless the object has a large space velocity. If the lifetime of these compact radio emitters is greater than that of the shell remnants, however, it is evident that there will be many without associated shells, as in the case of the radio pulsars. (5) Possible X-ray and/or optical counterparts.

In addition to the four sources already considered, Tables 1 and 2 list the properties of five other sources which have been found near SNRs and which we also regard as possible members of the new class. Some additional details of the sources, not previously mentioned, are as follows.

0125+628. From a small number of observations, Salter *et al.*⁴ detected no variations at 5 GHz, but it is clear from Fig. 1 that the flux density at 15.4 GHz is varying on a timescale of a month, and perhaps much faster, although the significance of the more rapid variations is not large. Figure 1 suggests that $\alpha_{2.7}^{15} \approx -0.2$ in 1977 May, and the flux values at 2.7, 8.1 and 10.6 GHz quoted by Shaffer *et al.*⁵ also confirm the flatness of the spectrum above about 2 GHz. There is a cut-off at lower frequencies.

0503+466. Point source in HB9, with $S(2.7 \text{ GHz}) = 590 \text{ mJy}$ (ref. 21). It is absent from the 4C records but has been detected in the 6C survey²² with $S(150 \text{ MHz}) = 740 \pm 250 \text{ mJy}$ in 1976 May (Masson, personal communication). Its spectrum is therefore very flat.

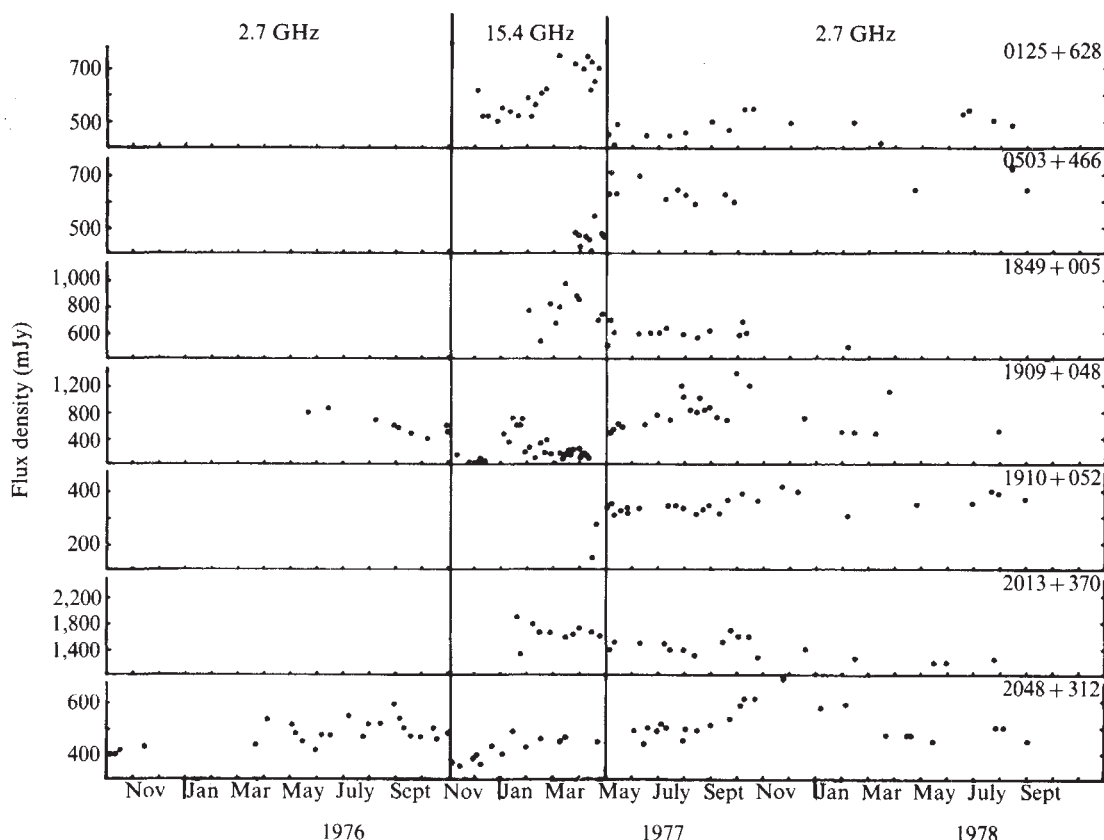


Fig. 1 Flux densities as measured with the 5-km telescope at 2.7 GHz (October 1975–October 1976, and May 1977–September 1978) and 15.4 GHz (November 1976–April 1977). Linearly polarised feeds were used with the E-vector in position angle 90°. The calibration assumed flux densities $S(2.7 \text{ GHz}) = 13.0 \text{ Jy}$ for 3C147, and $S(2.7 \text{ GHz}) = 10.3 \text{ Jy}$, $S(15.4 \text{ GHz}) = 3.69 \text{ Jy}$ for 3C286. The main contributions to errors are from receiver noise ($\sim 3 \text{ mJy}$ at 2.7 GHz, and 20 mJy at 15.4 GHz) and tropospheric variations ($< 3\%$ at 2.7 GHz, and $< 5\%$ at 15.4 GHz).

Table 2 Candidate flux densities and other data

Source	$S(1.4 \text{ GHz})$ mJy	Angular diameter 10^{-3} arc s	Spectral Index	Variable	SNR distance estimate (kpc)	Nearby SNR	Refs
0125+628	380	≈ 0.5	$\alpha_{2.7}^{15} = -0.2$	Yes	5.4	At centre of G127.1+0.5 (R5)	3, 5, 19, see text
0503+466	≈ 600	$< 150 \pm 130$	$\alpha_{2.7}^{15} \approx 0.2$	Yes	1.8	At edge of G160.5+2.8 (HB9)	See text
1347-617	230	$> 15\ 000$	$\alpha_{0.4}^{1.4} = 0.71$	?	8.1	Near centre of G309.8+0.0	See text
1516-569 (Cir X-1)	380	—	$\alpha_{0.4}^5 \approx 0.25$	Yes	8	Near G321.9-0.3	1, 17, 20
1849+005	≈ 800	$< 130 \pm 130$	$\alpha_{1.4}^{15} \approx 0.1$	Yes	9.4	Near G33.6+0.1	See text
1909+048 (W50X)	≈ 650	$< 150 \pm 100$	$\alpha_{0.4}^{15} \approx 0.1$	Yes	3.3	Near centre of G39.7-2.0 (W50)	See text
1910+052	≈ 600	$< 320 \pm 210$	$\alpha_{0.4}^{15} \approx 0.3$	Yes	3.3	Within G39.7-2.0 (W50)	See text
2013+370	1150	≈ 0.5	$\alpha_{2.7}^{15} \approx 0$	Yes	> 12	Near G74.9+1.2	See text; 19
2048+312 (CL4)	380	≈ 1	$\alpha_{1.4}^{15} \approx 0.21$	Yes	1.2	Within G74.0-8.6 (Cyg Loop)	2, 19

1347-617. This source is listed tentatively, on the grounds of its position very near the centre of the SNR G309.8+0.0. It contrasts with the other sources by apparently having a steeper spectrum, but flux variability might be responsible.

1849+005. This source was first noted by Caswell *et al.*²³ while mapping the SNR G33.6+0.1, and attention was also drawn to it as a possibly unusual galactic source by Caswell *et al.*²⁴. The spectral index quoted in Table 2 is based on a 5-km value of $S(15 \text{ GHz}) = 700 \text{ mJy}$.

1909+048. Figure 1 shows that this is highly variable, especially at 15 GHz. At epoch 1976.9, for example, $S(15 \text{ GHz}) < 20 \text{ mJy}$ but, within two months, $S(15 \text{ GHz})$ was 740 mJy.

1910+052. This second point source within the shell of W50 was first detected with $S(408 \text{ MHz}) = 780 \text{ mJy}$ in 1974. Like 1909+048, it has a small angular size, a flat spectrum and is almost certainly variable at 2.7 GHz (Fig. 1). There were two measurements at 15.4 GHz; these indicated a stronger variability but this requires confirmation. The flux density was also measured at Parkes, New South Wales as $S(5 \text{ GHz}) = 430 \text{ mJy}$ in May 1974 and $S(15 \text{ GHz}) = 230 \text{ mJy}$ in 1977 August. The occurrence of this second point source within a single SNR shell may be a chance superposition. It is clear that more statistical data on the spatial density of such point sources are required before we can evaluate this possibility.

2013+370. This region has been studied by Duin *et al.*²⁵ and Shaffer *et al.*⁵ The point source lies about 7 arc min outside the SNR boundary so, if the association is real, a high velocity of ejection ($> 5,000 \text{ km s}^{-1}$) would be necessary to account for the separation. HI absorption measurements²⁶ place similar lower limits (of $\sim 12 \text{ kpc}$) on the distance of both objects. Whereas the extended source is almost certainly a galactic SNR, present evidence does not rule out an extragalactic nature for the point source. The spectrum is, nevertheless, unusual by any standards, with a low frequency cut-off at the very high frequency of 7.5 GHz.

Discussion

Most of the sources investigated probably represent an important new class of radio star. The relationship of these sources to the other galactic X-ray sources and to pulsars is not yet clear although, if either 1909+048 or 1516-569 is indeed a member of the new class, then the other galactic X-ray binary systems (with or without detectable radio emission) may, in some cases at least, also be the stellar remnants of supernovae, perhaps having outlived their SNR shells.

It has been proposed²⁷ that all supernovae are formed in binary systems; this would be consistent with the X-ray binary stars originating in supernovae, and may indicate that the radio pulsars are generally confined to those binary systems which have been disrupted by the supernova explosion (in particular, the two pulsars definitely associated with SNRs are not binary

systems). If sources of the type discussed here are associated with binary systems not disrupted by the explosion, one may expect periodicities in the radio variations. Webster & Ryle², in fact, suggested that the flux variations of 2048+312 are quasi-periodic with a period of $\sim 60 \text{ d}$; we have carried out a power-spectrum analysis on all the available observations. This did not confirm the suggestion but, because of the irregularity of the observations, we cannot reject it either.

A major problem is that the radio properties of the new class are superficially similar to those of some extragalactic sources, the BL Lac objects, and it is indeed possible that some of our suggested examples of stellar remnants may be misidentified. Nevertheless, there can be no doubt that both types of source exist—some, such as the radio counterpart of Cir X-1, being definitely galactic, and others such as the BL Lac objects showing large redshifts, being definitely extragalactic.

We thank Dr B. Elsmore for providing the 5-km radio positions, C. R. Masson for the 150-MHz flux density of 0503+466, and Dr D. Wills for the optical position of the candidate identification for 0125+628, also Drs J. E. Baldwin, P. A. G. Scheuer and A. S. Webster for valuable comments.

Note added in proof—A spectrum²⁸ of the possible optical counterpart of 0125+628 reveals a faint galaxy. Thus if the radio-optical association is valid, this radio source is not galactic and its coincidence with the SNR is by chance. A red object²⁹ of 21 mag coincides with the radio source.

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K-Ar age of the late Pleistocene eruption of Toba, north Sumatra

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The late Pleistocene eruption of Toba is the largest magnitude explosive eruption documented from the Quaternary. K-Ar dating of the uppermost unit of the Toba Tuff gives an age of ~75,000 yr. A chemically and petrographically equivalent ash layer in deep-sea cores helps calibrate the Stage 4-5 boundary of the standard oxygen isotope stratigraphy. A similar ash in Malaya that overlies finds of Tampan Palaeolithic tools indicates that they are older than 75,000 yr.

A WIDESPREAD volcanic ash layer has been found in piston cores taken in the north-east Indian Ocean and the Bay of Bengal, where it occurs in the upper 12 m of sediment. This ash layer has been correlated from core to core on the basis of mineralogy¹. A study of the area distribution, thickness, grain size and volume of the deep-sea ash layer has shown that the ash originated in an eruption an order of magnitude larger than any other documented for the Quaternary² and indicates a source of the ash in an eruption of Toba, north Sumatra^{1,2}. Toba caldera, whose dimensions are 100 × 30 km, is surrounded by rhyolitic tuffs (Fig. 1) which occupy an area of 20,000–30,000 km² and have a thickness of several hundred metres (refs 3, 4). We report here on the radiometric dating of the Toba Tuff and the correlation of this eruptive.

Several samples of Toba tuffs have been dated. A sample from the base of the tuff series has been dated by the K-Ar method at 1.9 Myr (ref. 5). Three other samples have been dated by the fission-track method: samples of 'old tuff' and 'upper tuff' from

the Prapat area gave 1.2 and 0.1 Myr respectively. A third sample of 'welded tuff' from the area of Si Gura Gura gave an age of 0.1 Myr (ref. 6).

A K-Ar age of $75 \pm 12 \times 10^3$ yr on biotite has been reported⁷ from a Toba welded tuff sample Id 680. We report here the data on the K-Ar dating and on the chemical and mineralogical composition of sample Id 680 from Si Gura Gura and a sample of pumice tuff (72-0-60) from the Prapat area. We have also analysed chemically and mineralogically an ash layer which occurs in 15 deep-sea cores to the west and north-west of Toba (Fig. 1). We have established the position of the ash layer in one of the cores in the standard oxygen isotope stratigraphy, confirmed by two biostratigraphic data, to the extinctions of the radiolarian *Stylatractus universus* and coccolith *Pseudoemiliania lacunosa*. We have also analysed several samples of ash from the Kota Tampan area in west Malaya.

Toba tuffs

The welded tuff sample Id 680 (from the collection of Dr M. T. Zen) came from Si Gura Gura (Fig. 1), along the Asahan River, and was taken at a depth of 55 m.

The tuff consists of about 47% crystal fragments embedded in a matrix of fine crystalline material and volcanic glass. The composition of heavy minerals is given in Table 1. Only a few phenocrysts of allanite, reported by Westerveld⁸, have been identified in the studied sample. Opaque minerals are rare, and are mostly developed as secondary products on grain borders or within xenoliths. Xenoliths are abundant in the form of schist. The light mineral fraction was analysed using the methods described by Hayes and Klugman⁹ and the results plotted in Fig. 2. The volcanic glass has a refractive index of 1.496–1.500. Wet chemical analysis has been done on both the whole rock and pure glass shard fraction (Table 2). K-Ar dating was done on biotite and gave an age of $74.9 \pm 12 \times 10^3$ yr (Table 3).

Table 1 Composition of the heavy mineral fraction (%) in the Toba welded tuff and in the ash layer from Tampan and deep-sea cores (size fraction: 0.25–0.062 mm)

	Biotite	Hornblende	Hyperstene	Epidote	Muscovite	Tourmaline	Total (%)
Toba							
(Id 680)	86.0	12.0	2.0	—	—	—	100
RC14-37	84.0	16.0	—	—	—	—	100
RC14-38	72.0	27.5	0.4	—	0.1	—	100
RC17-145	79.0	20.0	1.0	—	—	—	100
Tampan							
6312	74.0	10.0	—	4.0	3.0	9.0	100
6313	85.0	3.0	0.3	0.7	5.3	5.7	100
6315	91.0	6.0	—	—	3.0	—	100
6317	86.0	7.0	—	—	3.6	3.4	100
	Pyrogenic			Detrital			

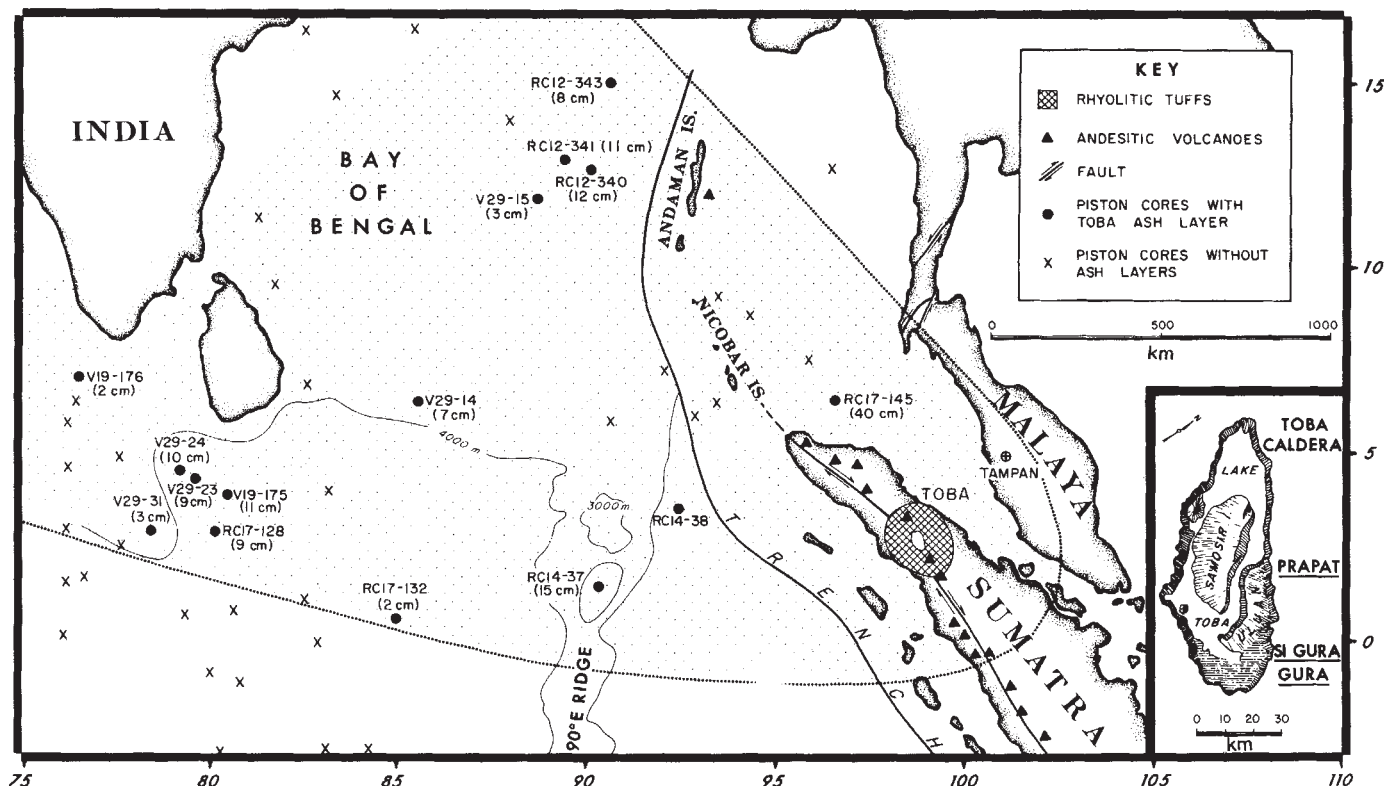


Fig. 1 Map showing the distribution of Toba tuffs on Sumatra and the equivalent ash layer in deep-sea cores and on Malaya (after refs 1 and 2). The inset shows the location of Si Gura Gura and Prapat to the east of Toba caldera, where the analysed samples of tuffs were collected.

Table 2 Chemical composition of the Toba welded tuff and the ash layers in deep-sea core and on Malaya

	1	2	3	4
SiO ₂	70.0	72.2	71.8	72.6
TiO ₂	0.37	0.11	0.14	0.16
Al ₂ O ₃	13.9	12.9	13.1	13.6
Fe ₂ O ₃	2.0	0.3	0.15	0.75
FeO	0.9	0.7	0.85	0.05
MnO	0.06	0.06	0.07	0.05
MgO	0.8	0.35	0.25	0.1
CaO	1.8	2.2	1.65	1.0
Na ₂ O	3.4	2.8	3.7	2.5
K ₂ O	5.2	5.1	5.1	4.6
P ₂ O ₅	0.06	0.03	0.05	0.06
H ₂ O	1.4	3.1	3.0	4.2
Total	99.89	99.85	99.86	99.67

Analyst M. Weibel.

1. TOBA (Si Gura Gura, Id 680); whole rock.
2. TOBA (Si Gura Gura, Id 680); glass shard fraction.
3. Deep-sea core RC14-37; ash layer at 100–115 cm, glass shard fraction.
4. Kota Tampan, Malaya; volcanic ash (UM6315); glass shard fraction.

The pumice tuff (72–0–60) sample was collected by J. D. Obradovich at Prapat (Fig. 1), where in 1972 an excavation above the lake exposed some 10 m of pumice tuff. The pumice fragments (5–10 cm) are predominantly glass with phenocrysts (2–4 mm) of quartz, sanidine, black biotite and brown hornblende; zircon was also recovered. No xenoliths are present.

K–Ar dating was done on sanidine and gave an age of $73.5 \pm 3 \times 10^3$ yr (Table 3). Because the radiogenic argon content for the analysis on sanidine is so much greater than that of the biotite analysis this result is considered to be the most reliable.

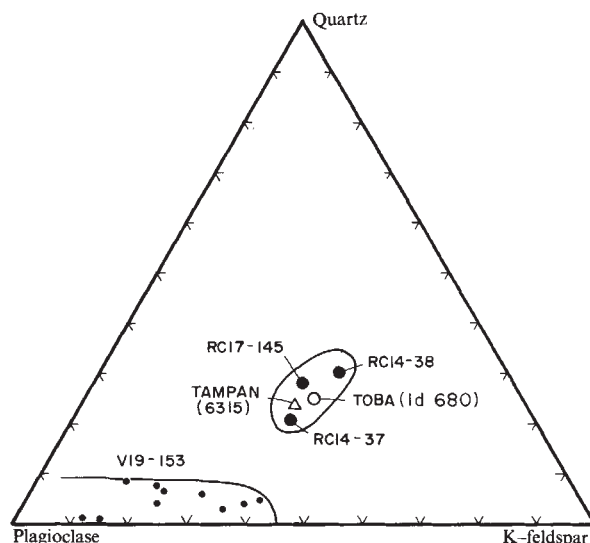
Toba ash layer in deep-sea cores

Fifteen deep-sea cores (Fig. 1) contain a single ash layer. Glass shards from the ash layer in all cores have a refractive index of 1.496–1.500, similar to that of the Toba tuff. Mineralogical composition of the deep-sea ash layer is also similar to the Toba sample (Table 1, Fig. 2). Chemical composition of the glass shard fraction from the ash in core RC14-37 is given in Table 2.

RC14-37 was taken from the top of the 90° E ridge. The ash layer, graded from the bottom upwards, occurs at 100–115 cm (Fig. 3). The core is composed of about 85% CaCO₃, and shows very little variation throughout the sediment.

Oxygen isotope measurements were made in *Globigerinoides*

Fig. 2 Correlation of upper Toba tuff with ash layer from Tampan, Malaya and deep-sea cores RC14-37, RC14-38 and RC17-145, based on light mineral composition.



sacculifer with a view to establishing the position of the ash layer in the standard oxygen isotope stratigraphy. The measurements (Fig. 3) indicate that the core extends to Stage 13 at about 0.5 Myr. This is confirmed by the observation that Stage 12 is bracketed by the extinction of *P. lacunosa* below¹¹, and *S. universus* above¹². The Toba ash layer is found at about the Stage 5–4 boundary (Fig. 3). The age of the Stage 5–4 boundary is generally agreed to be $\sim 75,000$ yr (ref. 13).

The recognition of the Toba ash in core RC14-37 and its K–Ar age of $73.5 \pm 3 \times 10^3$ yr allows an independent assessment

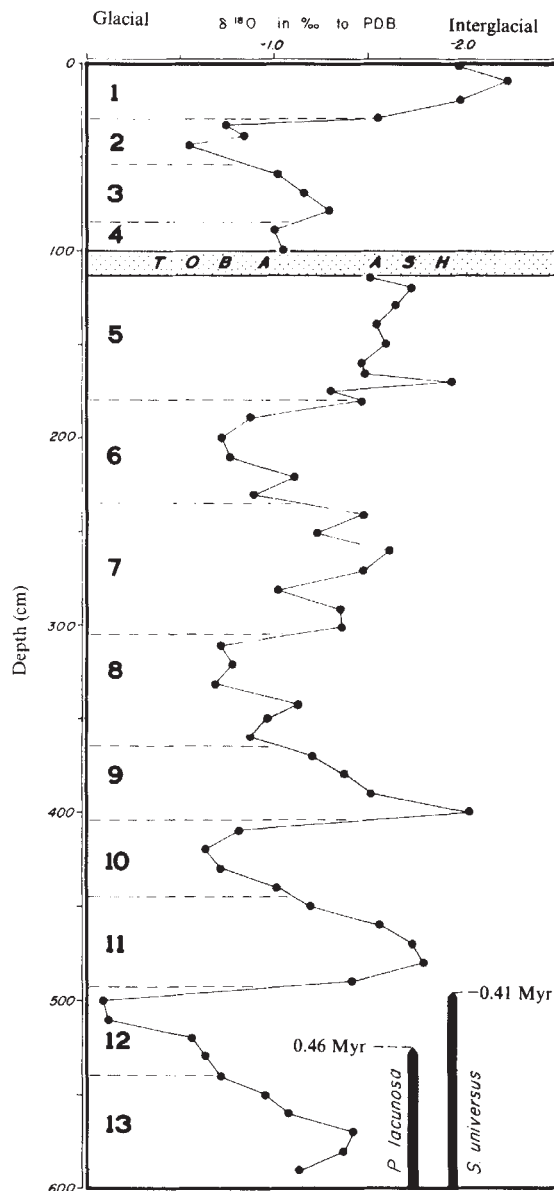


Fig. 3 Core RC14-37. Oxygen isotope curve, and coccolith *P. lacunosa* and radiolarian *S. universus* extinction levels (after refs 11 and 12). Toba ash occurs at about the $\delta^{18}\text{O}$ Stage 4–5 boundary.

of the age of the Stage 4–5 boundary. As the ages of the stage boundaries given by Shackleton and Opdyke¹³ are estimated on the basis of a uniform sedimentation rate in core V28-238 calibrated by the presence of the Brunhes–Matuyama magnetic epoch boundary, age 700,000 yr, our results confirm that this assumption is valid.

Volcanic ash from Kota Tampan

Volcanic ash, up to 3 m thick was first reported from the Kota Tampan area in west Malaya by Scrivenor¹⁴ and suggested by van Bemmelen⁴ to have originated in the Toba eruption. This

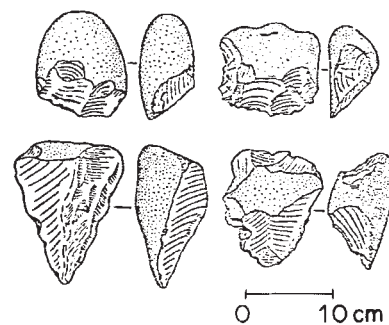


Fig. 4 Tampan Palaeolithic tools and their stratigraphic position (after ref. 15).

ash has received much attention, particularly because it covers gravels from an ancient river terrace in which Palaeolithic tools have been found¹⁵ (Fig. 4). The tools have been studied archaeologically by Collings¹⁵, Sieveking¹⁶, and more recently by Harrison¹⁷, who questions the validity of the Tampan artefacts.

An age of about 35,000 yr for the Tampan Palaeolithic tools, attributed by Harrison, is based on ^{14}C dating of wood reported by Stauffer¹⁸. Three samples of wood found under the ash have been dated and gave the following ages: $33,250 \pm 1,800$, $36,500 \pm 2,500$, and greater than 39,000 yr BP. One sample of wood found above the ash layer gave an age of $1,145 \pm 90$ yr BP (ref. 18). In view of our results, however, it seems likely that the two samples that yielded apparently finite ^{14}C ages were contaminated by recent carbon (see Stuiver et al.¹⁹ for an up to date discussion of contamination of samples by modern carbon).

Several samples of Tampan ash were provided by N. Haile. The following four samples have been analysed: UM6312 and 6313 ($100^{\circ}58'40''\text{E}$, $5^{\circ}3'30''\text{N}$), UM6315 ($100^{\circ}57'50''\text{E}$, $5^{\circ}3'25''\text{N}$) and UM6317 ($100^{\circ}57'30''\text{E}$, $4^{\circ}49'10''\text{N}$). Volcanic glass shards from all samples have a refractive index similar to that of the Toba tuff (Id 680). Chemical composition of the

Fig. 5 Electron microprobe analysis of *a*, volcanic glass; and *b*, sanidine from upper Toba tuff and from volcanic ash on Malaya (analyst G. Izett). ●, Toba (72–0–60); ×, Malaya (UM 6313).

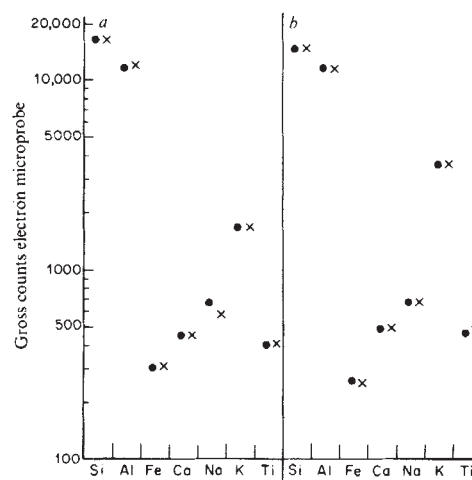


Table 3 Analytical data for Toba tuffs

Sample	Mineral	% K	mol $^{40}\text{Ar}^*$ per g $\times 10^{-12}$	% Rad Ar	Age (10^3 yr)
Si Gura Gura Id 680§	Biotite	6.17†	0.959 0.634 av. 0.797	1.60 0.36	74.9 $\pm$ 12
72-0-60 Prapat	Sanidine	9.88‡	1.259	13.1	73.5 $\pm$ 3

Decay Constants: $^{40}\text{K}/\text{K} = 1.167 \times 10^{-4}$ atom/atom; $\lambda_B = 4.962 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_{e+e'} = 0.581 \times 10^{-10} \text{ yr}^{-1}$ (from Steiger and Jaeger¹⁰).

† Potassium by atomic absorption.

‡ Potassium by isotope dilution.

§ Analyst A. A. Abdel-Monem.

|| Analyst J. D. Obradovich.

samples is given in Table 1 and relative proportions of light minerals in Fig. 2.

The relative proportions of light minerals in sample UM6315 is similar to that of the Toba tuff and the deep-sea ash layer. The chemical composition of the pure glass shard fraction of sample UM6315 is given in Table 2. The three other samples have a higher quartz content, and contain a small per cent of tourmaline, suggesting contamination by residual sand.

Electron microprobe analysis for seven elements has been done on volcanic glass shards and sanidine from the Tampan sample UM6313 and Toba sample 72-0-60 and those data are plotted as gross counts in Fig. 5. The match for these seven elements is exceedingly good. The sanidine phenocrysts of both samples are notable in that they have an extremely high value for K_2O of 11.9% based on isotope dilution analysis of sample 72-0-60.

Volcanic ash layers in deep-sea cores near Sunda Strait

Several piston cores containing rhyolitic ash layers have been taken to the south-west of Sunda Strait²⁰, a few hundred kilometres from the rhyolitic tuff fields of south Sumatra and west Java. Core V19-153 contains 10 ash layers, whose age and chemical composition have been reported by Ninkovich¹.

The compositions of the light mineral fraction from 10 ash layers in core V19-153 are shown in Fig. 2, for comparison with the Toba products. It is obvious from these data that the source of the ash in Malaya and in core RC14-37 is in Toba and not the same as the source of the ash in the more southerly cores.

Conclusion

Sample Id680 from Si Gura Gura taken near the top of the Toba tuffs and the ash layer in deep-sea core RC14-37 have a similar chemical and mineralogical composition. K-Ar dating of the Toba tuff sample Id 680 from Si Gura Gura and sample 72-0-60 from Prapat, and oxygen isotope and biostratigraphic dating of

the ash layer in core RC14-37 have provided a similar estimate of about 75,000 yr for the age of the eruption. Examination of core RC14-37 indicates that the 75,000 BP eruption was the only major explosive eruption of Toba in the past 0.5 Myr. Chemical and mineralogical composition of the thick Tampan ash suggests its source was also the 75,000 yr old Toba eruption. The K-Ar age of $73.5 \pm 3 \times 10^3$ yr obtained for sanidine from the eruption provides a valuable independent check on the age of the Stage 5-4 boundary in the standard oxygen isotope stratigraphy.

Toba tuff sample Id 680 was provided by Dr M. T. Zen, and ash samples from Malaya by Professor N. Haile. The manuscript was reviewed by F. McCoy and L. H. Burckle. The research was partly funded by NSF grant OCE-76-80690. The core curating facilities at LDGO are supported by the Office of Naval Research (contract N00014-75-C-0210-Scope E) and the NSF (contract OCE-76-18049).

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Asymmetric gene conversion at inserted segments on yeast mitochondrial DNA

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Different molecular weight forms of the protein product of the yeast mitochondrial gene var1 are shown to arise by a process of asymmetric gene conversion. These different forms can be accounted for by two DNA segments, ~36 and 57 base pairs long, present in one allelic form of the var1 structural gene, which can be inserted independently and at different frequencies into other var1 alleles.

THE mitochondrial genome of bakers' yeast *Saccharomyces cerevisiae* has been the subject of intensive genetic and biochemical investigation. The genome is apparently highly active in genetic recombination and many phenomenological aspects of the recombination, transmission and segregation of mitochondrial genes have now been well defined (see ref. 1 for a review). However, there is still little information available concerning the molecular mechanisms underlying these processes.

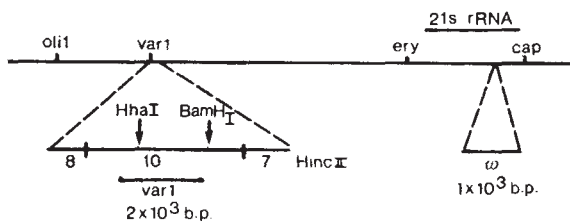


Fig. 1 Diagrammatic representation of the *cap-oli1* region of the yeast mitochondrial genome, showing the relative locations of the *var1* gene and the ω insertion, to markers for chloramphenicol resistance (*cap*^r), erythromycin resistance (*ery*^r) and oligomycin resistance (*oli1*). *Cap* and *ery* are located in the region encoding the 21S mitochondrial ribosomal RNA; this region is separated from *oli1* by a distance of 11×10^3 base pairs. The determinant ω , an insertion of 1,000 base pairs^{10,11}, is located within the 21S ribosomal DNA segment. Yeast strains whose mtDNA contains the insertion are termed ω^+ ; those whose mtDNA lacks it, ω^- .

ses. We know that in most cases, mitochondrial genetic markers are transmitted coordinately to the diploid progeny of crosses. Furthermore, markers tend to segregate rapidly during vegetative growth and for most alleles, reciprocal recombinant genotypes are recovered in equal numbers²⁻⁶. A well established exception to this last point involves a determinant located in the 21S ribosomal DNA region of the mitochondrial genome designated ω (refs 7, 8) (Fig. 1). Crosses having the configuration $\omega^+ \times \omega^-$ (termed 'heteropolar') produce grossly unequal numbers of genotypes recombinant for the genetic markers in the vicinity of the ribosomal DNA region⁷⁻⁹. Physical mapping studies of mitochondrial DNA (mtDNA) in yeast strains with ω^+ and ω^- alleles show that mtDNA from ω^+ strains has an insertion of 1,000 base pairs near the markers *cap* and *ery* (Fig. 1) that is absent from mtDNA of ω^- strains^{10,11}. The major consequence of this difference seems to be a polarity of recombination in the ribosomal DNA region as a result of gene

conversion events in the direction ω^- to ω^+ . Thus, the 1,000-base pair insertion spreads in heteroplasmic cells among the population of mtDNA molecules. In homopolar crosses, where both parents have the same ω allele, either ω^- or ω^+ , no polarity of recombination is observed⁸.

Several other strain-specific differences involving various combinations of insertions and deletions in wild-type (ρ^+) mtDNA have been noted^{11,12}. These seem to involve DNA segments from <100 to >1,000 base pairs. Fonty *et al.*¹³ have reported that when two ρ^+ strains differing in *HaeIII* and *HpaII* restriction endonuclease fragment patterns are mated, a portion of the diploid progeny have one or more restriction fragments that are non-parental. It was suggested that these 'new' bands arise as a result of unequal crossing-over in dAT 'spacer' sequences known to comprise about 50% of the mtDNA in *S. cerevisiae*¹⁴.

We have previously described a product of mitochondrial protein synthesis with novel properties¹⁵. When mitochondrially synthesised proteins from different ρ^+ yeast strains were analysed on SDS-polyacrylamide gels, most of the major mitochondrial translation products (three subunits of cytochrome oxidase, and the presumed apoprotein of cytochrome *b*) had identical mobilities in all strains. However, a protein termed *var1*, not previously associated with any mitochondrial function, was found to vary in electrophoretic mobility among the different ρ^+ strains analysed. Thus far, we have identified at least six strain-dependent forms of the *var1* polypeptide ranging in molecular weight from 40,000 to 44,000 which are stable during vegetative growth¹⁶. This polymorphism was used as a genetic marker to show that the structural gene for the polypeptide is located on mtDNA between the markers *ery* and *oli1* (ref. 17) (Fig. 1). By a combination of petite deletion mapping, zygotic gene rescue¹⁸, and restriction endonuclease analysis of ρ^+ and petite mtDNA samples, we have located the structural gene for the *var1* polypeptide to a DNA fragment about 2×10^3 base pairs in length, beginning 9.7×10^3 base pairs from a unique *SaII* site on mtDNA in ω^- strains and 10.7×10^3 base pairs from the same *SaII* site in ω^+ strains¹⁶ (R.D.V. *et al.*, unpublished data). An important conclusion drawn from our studies was that the variant forms are the result of sequence length differences in the *var1* structural gene itself.

In this report we present a detailed analysis of the origin of different forms of *var1* polypeptide. The results provide a satisfying explanation for the apparent widespread polymorphism exhibited by this protein and may be of general significance concerning genetic exchanges in yeast mtDNA. The data demonstrate, at the molecular level, a process of asymmetric intragenic gene conversion which can occur in crosses at a frequency at least equal to that of the observed upper limit of recombination between unlinked mitochondrial markers⁴. The gene conversion events can result in either the conversion of one parental allele to the other, or the formation of new, non-parental forms of the *var1* gene. Although these events at *var1* are assayed differently from those at ω , there is a great similarity between the types of gene conversion exhibited at these two regions of the mitochondrial genome.

Assay for *var1* polypeptides in mixed cell populations

In our previous genetic studies, the distribution in crosses of different forms of *var1* polypeptide was studied by first isolating homoplasmic diploids and then analysing the individual diploid clones for their drug resistance and *var1* alleles^{16,17}. Because the *var1* allele must be determined by analysis of mitochondrial translation products on SDS-polyacrylamide gels, the procedure becomes quite cumbersome for scoring *var1* transmission in a large number of crosses. Consequently, to facilitate the studies described here, we determined whether it was possible to measure quantitatively the relative amounts of different molecular weight forms of *var1* polypeptide in mixed cell populations.

To this end, we carried out a reconstruction experiment in which two isonuclear diploid clones, one of genotype *cap*^r *var1*

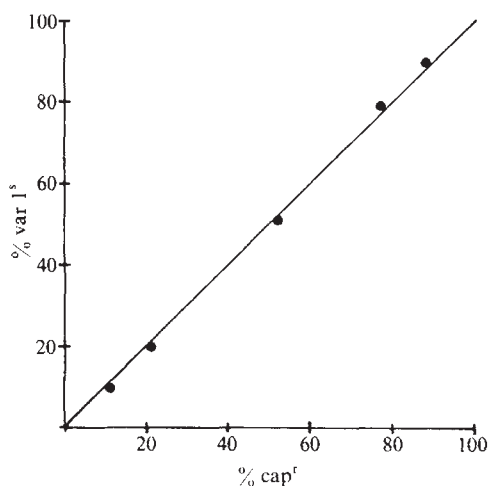


Fig. 2 Calibration curve for an assay of the ratio of *var1* alleles in mixed cell populations. Two diploid segregants of genotype *cap*^r *var1*^S (Mw 44,000) and *cap*^r *var1*^F (Mw 40,000) were obtained from the cross 5DSS (*cap*^r *var1*^S) $\times$ 195D (*cap*^r *var1*^F). These were grown to log phase at 28°C in YEP medium (1% yeast extract, 1% bacto-peptone) containing 2% dextrose and were then mixed in various proportions. To determine the exact percentage of each cell type in the mixed cell population, an aliquot was plated on YEP medium containing 2% dextrose and then replica plated on to YEP medium containing 3% glycerol and 3 mg ml⁻¹ chloramphenicol. Mitochondrial translation products of each mixed cell population were labelled *in vivo* with ³⁵SO₄²⁻ and analysed on SDS-polyacrylamide gels according to the procedure of Douglas and Butow¹⁵. The autoradiogram of the SDS-polyacrylamide gel was traced with a quick scan densitometer (Helena) and the gaussian curves representing *var1* polypeptide were quantitated with a Dupont curve resolver.

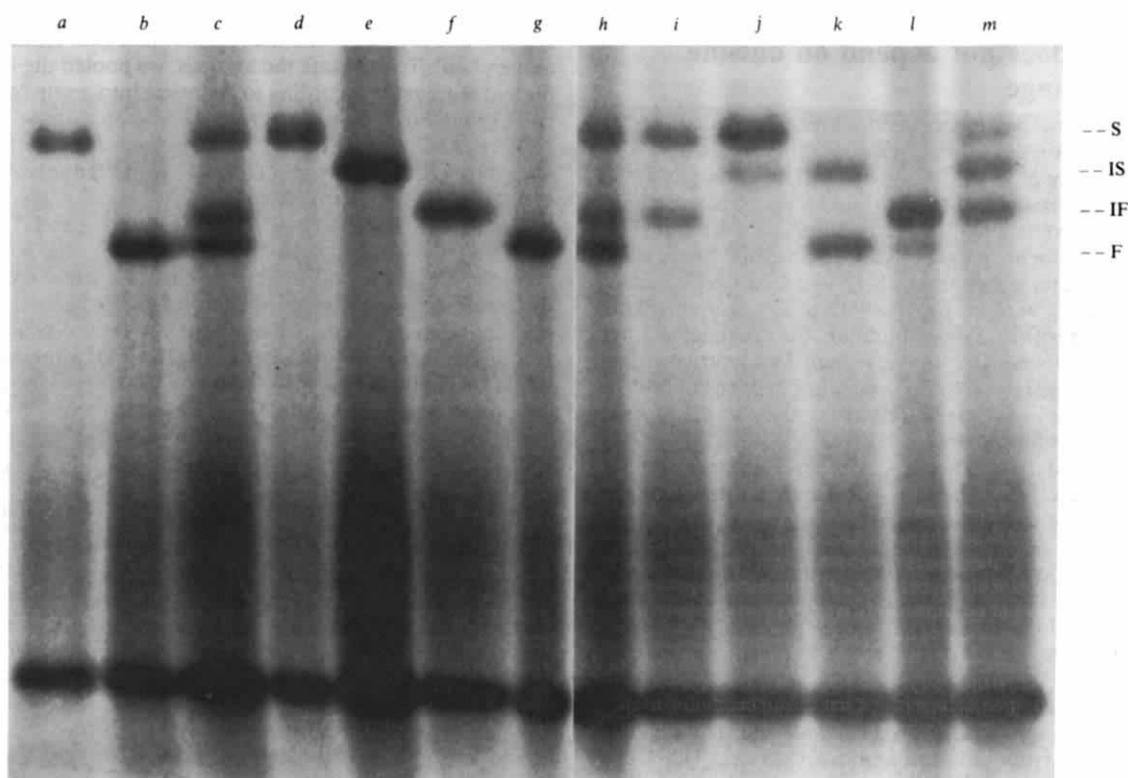


Fig. 3 Analysis of *var1* polypeptide in crosses. Wild-type strain 5DSS ($ura^+ \omega^+ cap^s ery^+ oli^s var1^S$) and 195D ($\alpha ade lys \omega^+ cap^r ery^s oli^r var1^F$) were mated. Random diploid⁷ as well as homoplasmic diploid clones were obtained and their mitochondrial translation products analysed as indicated in Fig. 2. The *var1* alleles for the haploid strains 5DSS and 195D are shown in lanes *a* and *b*, respectively. Random diploid progeny from the cross are shown in lane *c*. Homoplasmic diploid segregants containing the four progeny *var1* types (*var1*^S, *var1*^{IS}, *var1*^{IF}, and *var1*^F) are shown in lanes *d*–*g*, respectively. These diploid segregants were sporulated and haploid products representing each of the four *var1* forms were obtained with the appropriate nuclear markers so that all possible pairwise crosses could be carried out. In addition, the mitochondrial marker configuration in each case was such that the *ery* and *oli1* alleles could be scored. Lanes *h*–*l* represent the mitochondrial translation products of a sample of random diploid progeny issued from the following crosses: lane *h*, *var1*^F × *var1*^S; lane *i*, *var1*^S × *var1*^{IF}; lane *j*, *var1*^S × *var1*^{IS}; lane *k*, *var1*^{IS} × *var1*^F; lane *l*, *var1*^{IF} × *var1*^F; lane *m*, *var1*^{IF} × *var1*^{IS}.

(Mw 44,000) and the other *cap*^s *var1* (Mw 40,000), were mixed in different ratios and each mixed cell population labelled with ³⁵SO₄²⁻ in the presence of cycloheximide and products of mitochondrial protein synthesis displayed on SDS–polyacrylamide gels. Densitometer tracings of autoradiograms of the gel were analysed to determine the ratios of the two different molecular weight forms of *var1* polypeptide; these were then compared to the input ratios of cell types determined by the proportion of *cap*^r to *cap*^s cells. As shown in Fig. 2, there is an excellent linear relationship between the proportion of the two cell types as determined by the *cap* marker and the proportion of *var1* polypeptides, thus validating the assay of different forms of the protein in mixed cell populations.

Table 1 The generation of new forms of *var1* polypeptide does not depend on outside marker (*ery*, *oli1*) exchange

Genotype	% of total diploids	% of output <i>var1</i> ^S + <i>var1</i> ^F	<i>var1</i> ^{IF}
Parental (<i>ery</i> ^r <i>oli</i> ^s + <i>ery</i> ^s <i>oli</i> ^r)	71	68	32
Recombinant (<i>ery</i> ^s <i>oli</i> ^s + <i>ery</i> ^r <i>oli</i> ^r)	29	73	27

Homoplasmic diploid segregants were isolated from the cross 5DSS ($\omega^+ ery^r oli^s var1^S$) × 195D ($\omega^+ ery^s oli^r var1^F$) and scored for the *ery* and *oli1* loci. 200 segregants of each parental (*ery*^r *oli*^s, *ery*^s *oli*^r) and recombinant (*ery*^r *oli*^r, *ery*^s *oli*^s) genotypes were obtained and the percentage of each *var1* type among the progeny was quantitated using a mixed population assay (Fig. 2).

Non-parental forms of *var1* polypeptide appear in the progeny of some crosses

Analysis of the transmission of the *var1* gene in crosses between strains with different *var1* alleles led to the unexpected finding that in some crosses, progeny with non-parental forms of *var1* polypeptide are obtained. The most striking example is seen in the random diploid progeny from the cross between strains 5DSS and 195D (Fig. 3) which carry, respectively, the largest (44,000) and the smallest (40,000) molecular weight forms of the protein detected thus far. For ease of discussion, we refer to the 44,000 molecular weight species as the S (slow) form and the 40,000 molecular weight species as the F (fast) form.

In the random diploid progeny obtained from this cross, about 30% of the total *var1* polypeptide occurs as a non-parental form, with a mobility intermediate between the two parental types, corresponding to an apparent molecular weight of 41,800 (Fig. 3). We subcloned the mixed progeny of the cross and scored *var1* alleles in 60 homoplasmic diploid clones. In addition to the two parental and the 41,800 molecular weight forms, we found two clones with another non-parental form of *var1* polypeptide of molecular weight 42,200. Examples of the two parental and two non-parental types of homoplasmic diploid progeny are shown in Fig. 3. These new forms of *var1* polypeptide are designated IF (intermediate, fast) for the 41,800 species and IS (intermediate, slow) for the 42,200 species. These are the only non-parental forms of *var1* polypeptide that we have observed among 300 individual diploid progeny analysed from this cross.

The generation of new forms of *var1* polypeptide does not depend on outside marker exchange

For most mitochondrial genes, separation of pairs of alleles by roughly 10,000 base pairs or more is sufficient to yield a recombination frequency of 20–25%, the upper limit generally being observed. The formation of IF is striking, as it occurs at about that frequency even though the structural gene is contained within a DNA segment no greater than about 2,000 base pairs¹⁶ (R.D.V. *et al.*, unpublished data). If IF and IS arise by recombination, we could account for the data of Fig. 3 by invoking high-frequency reciprocal recombination, for example, at a hot spot within the gene. Alternatively, high-frequency unequal crossing-over or a gene conversion event in which one or both of the parental forms is converted to the new allele at a high rate, could also account for the data.

Because IF and IS are not obtained in equal proportions (about 30% and 2%, respectively) it is unlikely that they are mainly the products of a reciprocal recombination event; this conclusion is supported by the observation that in mixed cell cultures, there is no difference in the growth rates of cells that correlates with *var1* genotype. Unequal crossing-over is also an unlikely mechanism to account for our results as we have never observed new forms of *var1* polypeptide in crosses of the type F × F or S × S.

We have examined whether the generation of non-parental forms of *var1* polypeptide depends on outside marker exchange. We repeated the cross between strains 5DSS and 195D and selected homoplasmic diploid segregants for each of the four possible genotypes involving the *ery* and *oli1* drug markers

which flank the *var1* locus. These are the parental genotypes *ery*^r *oli*^s and *ery*^s *oli*^r 1 and the recombinant genotypes *ery*^r *oli*^r 1 and *ery*^s *oli*^s. To facilitate the analysis, we pooled the individual diploid segregants according to genotype into groups of 20. Ten such groups (of each genotype) were then analysed for the relative distribution of the S, F and IF forms of *var1* polypeptide. IS is not generated at a sufficiently high frequency to allow quantitation in this experiment.

The data in Table 1 show that IF comprises about the same fraction of the total *var1* species in either the parental or recombinant classes of progeny. Hence, we can conclude that the generation of IF is independent of any reciprocal crossing-over between its flanking markers. We have also observed that recombination at the *var1* locus is unaffected by interactions of ω^+ and ω^- in heteropolar crosses (data not shown); however, all the crosses reported here are of the homopolar ($\omega^+ + \omega^+$) type.

Non-parental forms of *var1* polypeptide arise by gene conversion

Gene conversion can be detected as the loss of one parental allele with a concomitant increase in recovery of the other parental allele or of a recombinant form. To determine if new forms of *var1* polypeptide arise by a process of gene conversion, we compared the transmission of the parental alleles with that of the *ery* and *oli1* drug markers. Because, in homopolar crosses, drug markers are recovered coordinately among the progeny, any gene conversion events involving *var1* could be detected as a deviation from coordinate transmission with *ery* and *oli1*. As shown in Table 2 (cross 1) (see Fig. 3), S was transmitted, within experimental error, coordinately with *ery*^r and *oli*^s. On the other

Fig. 4 *HincII* + *Bam*HI restriction endonuclease pattern of mtDNA. mtDNA was prepared from ρ^+ strains 5DSS, 195D and recombinant diploid progeny derived from their mating (see Fig. 3). The mtDNA of each strain was restricted with *HincII* + *Bam*HI and electrophoresed on a 2% agarose slab gel for 890 V h, stained for 10 min with 0.5 mg ml⁻¹ ethidium bromide and photographed under UV illumination (256 nm). In these electrophoretic conditions, the small *Bam*HI fragment generated from *HincII*-10 is not shown because it runs off the gel. Lanes *a* and *h*, Φ X174-RF DNA (combination of digests with *Hpa*II and *Hae*III); lanes *b*-*g*, mtDNA digested with *HincII* + *Bam*HI; lane *b*, strain 5DSS; lane *c*, diploid *var1*^S; lane *d*, diploid *var1*^{IS}; lane *e*, diploid *var1*^{IF}; lane *f*, diploid *var1*^F; lane *g*, strain 195D. The arrow indicates the large *HincII*-10 + *Bam*HI fragment.

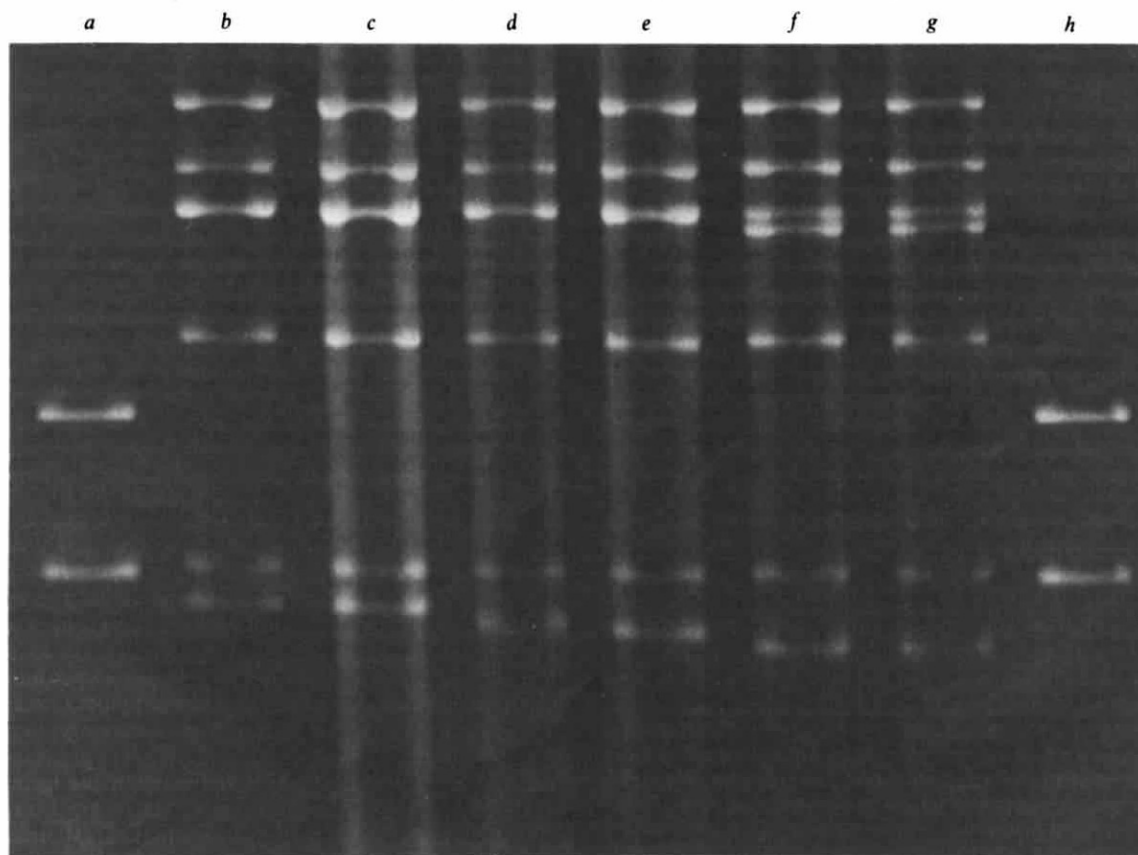


Table 2 Quantitative transmission of *var1* and antibiotic markers in crosses

Cross	<i>ery^s oli^r1</i>	×	<i>ery^r oli^s</i>	<i>ery^s, oli^r1</i>	<i>ery^r, oli^s</i>	% Transmission			
						<i>var1^F</i>	<i>var1^{IF}</i>	<i>var1^{IS}</i>	<i>var1^S</i>
1	F	×	S	53	47	27(53)	30	2	40(47)
2	F	×	IF	60	40	29(60)	71(40)	—	—
3	F	×	IS	62	38	61(62)	—	39(38)	—
4	IF	×	S	52	48	—	51(52)	—	49(48)
5	IS	×	S	43	56	—	—	21(43)	79(56)
6	IF	×	IS	38	62	—	39(38)	39(62)	22

The progeny of crosses described in Fig. 3 were scored for transmission of the *var1*, *ery* and *oli* loci. *Var1* transmission was quantitated according to the protocol described in Fig. 2. In each case, the cross was repeated several times and the values given are means. The expected transmission values for *var1* based on coordinate transmission with the drug markers, are given in parentheses. The dash indicates that no *var1* of that type was observed in random diploid progeny.

hand, compared to the transmission of *ery^s* and *oli^r1*, only about one-half of the input F alleles were recovered in the output of the cross. Importantly, this loss could largely be accounted for by the amount of the IF species which occurred in the diploid progeny, suggesting that this new form of *var1* arises chiefly by a gene conversion of the F allele. As the frequency of production of the IS species is less than 2%, it is difficult from this type of experiment to determine whether it also arises by a gene conversion event. Although the transmission of S is within two standard deviations of that of *ery^r* and *oli^s*, every estimate of S transmission was numerically smaller than that of the drug makers; thus, it is possible that there is some net loss of S so that reciprocal recombination could play a minor part in the generation of non-parental forms of *var1*.

To examine this possibility and to determine whether new forms arise only when parental genomes pair, diploids representing each of the four forms of *var1* polypeptide were sporulated and the appropriate mating types and nuclear markers obtained in the haploid products to permit all possible pairwise matings involving the four forms of the *var1* polypeptide. In Table 2, cross 2, we see that when F and IF are mated, both *var1* alleles are transmitted non-coordinately with the drug markers; we observed low transmission of F and high transmission of IF. This result helps to explain the frequency of IF production in cross 1 (Table 2), as IF genomes formed in the first round of recombination between parental genomes can pair with F genomes to yield more IF in subsequent pairings.

The results of all the other pairwise crosses are shown in Fig. 3 and Table 2, crosses 3–6. The results were clearcut in that no gene conversion was observed in the crosses F×IS or IF×S, whereas conversion was observed in the crosses IS×S and IF×IS. In the latter two cases, IS was converted to S. Note that in the case of cross 6, the conversion of IS to S represents, insofar as this particular cross is concerned, the formation of a non-parental form of *var1*. This result shows clearly that the gene segments which distinguish IF from IS are non-overlapping and leads to the conclusion that F and S differ from each other in at least two distinct regions.

As indicated above, IS could arise in cross 1 by gene conversion or by crossing-over. If the latter were true, then the frequency of crossing-over within the *var1* gene would be of the order of 2%, that is, the frequency of occurrence of IS in cross 1. This would mean that in cross 6, F should then be present in roughly 2% of the total progeny. To obtain greater sensitivity, we analysed an additional 200 random diploid progeny of cross 6 in 20 pooled groups of 10. Again, no F was detected, suggesting that if there are any F progeny in cross 6, they are produced less frequently than IS in cross 1. These data strongly suggest that in the original F×S cross, IS arises by a gene conversion event, but one less frequent than that responsible for the production of IF.

Non-parental forms of *var1* arise by physical exchange of DNA sequences

We next extended our analysis to the segment of mtDNA containing the *var1* gene. As noted above, we have succeeded in locating the *var1* gene to a specific *HincII* + *BamHI* restriction

endonuclease fragment, about 2×10^3 base pairs long located between *ery* and *oli1* (Fig. 1), the absolute size of which is strain dependent and varies according to the size of the *var1* polypeptide which it encodes¹⁶ (R.D.V. *et al.*, unpublished data).

We isolated mtDNA from homoplasmic diploid progeny of cross 1 (Table 2) representative of each of the four forms of *var1* polypeptide and digested it with *HincII* and *HincII* + *BamHI*. As indicated in Fig. 1, *HincII* fragment 10 (*HincII*-10) contains one *BamHI* site. The structural gene for *var1* polypeptide is contained wholly within the larger *HincII*-10 + *BamHI* fragment¹⁶ (R.D.V. *et al.*, unpublished data). Figure 4 shows representative restriction digests of mtDNA from each of the four diploid clones with the parental and non-parental forms of *var1* polypeptide. The gel was electrophoresed long enough to accentuate the difference between the large *HincII*-10 + *BamHI* fragments. The small *HincII*-10 + *BamHI* fragment was run off the gel; its molecular weight has been determined on separate gels run for a shorter time. The data show quite clearly that the large *BamHI* fragment of *HincII*-10 varies in size according to the size of the *var1* polypeptide for each strain with a different molecular weight form of the protein.

These results are summarised quantitatively in Table 3, which contains our estimates of the molecular weights in the different strains of the *HincII*-10 and *HincII*-10 + *BamHI* fragments. Within experimental error, the sizes of the large *BamHI* fragment of *HincII*-10 in the diploids with the S and F forms of *var1* polypeptide are the same as those in the parental haploid strains 5DSS and 195D, respectively. The fragment is larger in IS than in IF strains and both are intermediate in size between the S and F forms. Without exception, therefore, a change in the size of *var1* polypeptide is accompanied by a change in the size of the mtDNA fragment containing its structural gene.

We have also analysed mtDNA from progeny of the IS×IF cross where the parental S form is generated (Fig. 3). In this case,

Table 3 Apparent molecular weights of mtDNA fragments from ρ^+ strains with different *var1* alleles

Strain	<i>HincII</i> band 10	<i>HincII</i> band 10 + <i>BamHI</i>	
		Large fragment	Small fragment
5DSS	1.872 ± 0.006	1.438 ± 0.005	0.428 ± 0.001
S	1.842 ± 0.008	1.435 ± 0.007	0.427 ± 0.001
IS	1.842 ± 0.007	1.409 ± 0.009	0.427 ± 0.003
IF	1.813 ± 0.004	1.395 ± 0.007	0.406 ± 0.002
F	1.788 ± 0.009	1.376 ± 0.008	0.405 ± 0.002
195D	1.790 ± 0.008	1.377 ± 0.008	0.406 ± 0.002

mtDNA from ρ^+ strains 5DSS and 195D and recombinant diploid progeny containing parental (S, F) or non-parental (IS, IF) forms of *var1* polypeptide were analysed as in Fig. 3. Apparent molecular weights of *HincII* band 10, or the fragments generated from it by digestion with *BamHI*, were calculated by comparison with the mobilities of Φ X174-RF DNA fragments (combination of digests with *HaeII*, *HaeIII* and *HpaII*) run in adjacent lanes on slab gels containing 2% agarose. *HincII* digests were run for 850–900 V h; *HincII* + *BamHI* digests for 630 V h to measure the small fragment of *HincII* band 10, or 850–900 V h to measure the large fragment. The small fragment runs off the gel when maximal separation of the large fragments is obtained. Each value represents the mean of 4–7 independent gel measurements.

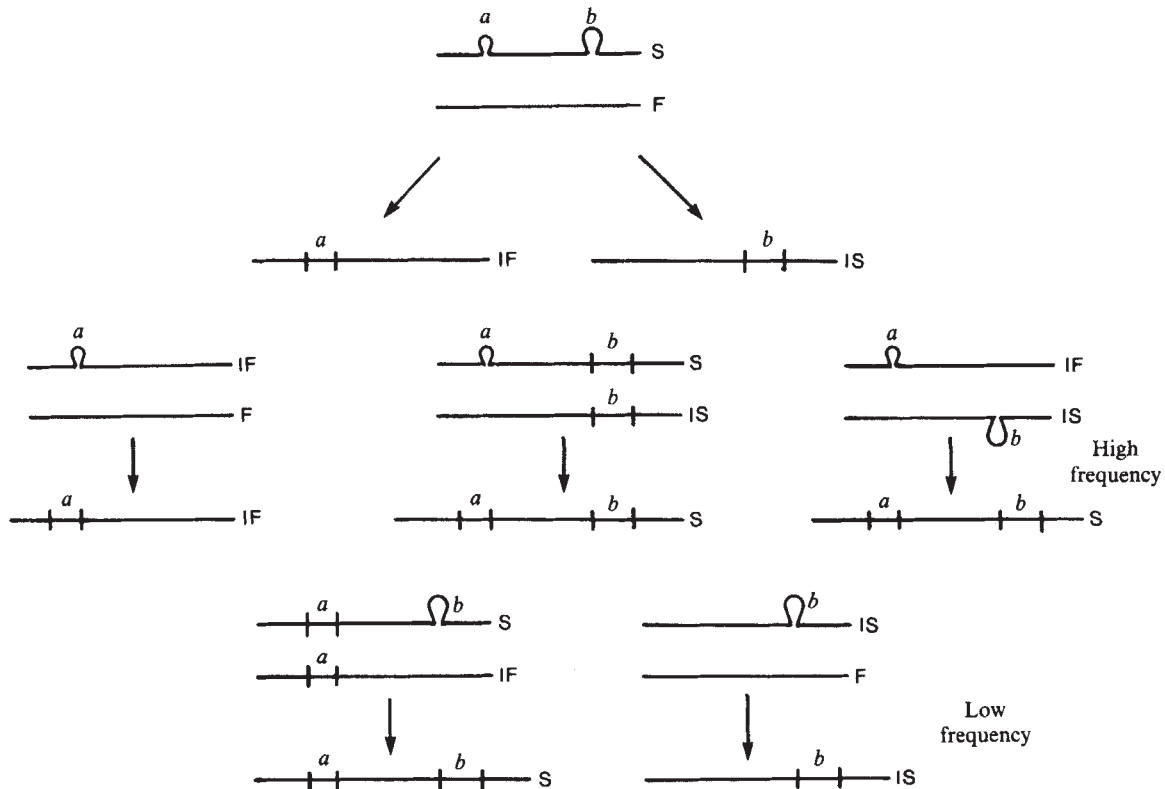


Fig. 5 A model for asymmetric gene conversion at the *var1* locus. Heteroduplex pairing of DNA segments from different *var1* alleles is shown; the positions of the looped out portions are arbitrary.

the size of the *HincII*-10+*Bam*HI large fragment in the S diploids issued from this cross is indistinguishable from the parental (5DSS) S form (data not shown). This result, and the fact that the sum of the molecular weight differences between IF, IS and F equals the molecular weight difference between S and F, provide additional support to our conclusion that the regions of difference between IF and IS are non-overlapping.

A model for gene conversion at the *var1* locus

Figure 5 presents a simple scheme fully consistent with the genetic and biochemical data presented here, showing how non-parental forms of the *var1* gene and its product may arise by a process of asymmetric intragenic gene conversion. The model shows two DNA segments or insertions within the S gene which are absent from the F gene. From molecular weight determinations of the parental and non-parental DNA fragments which contain the *var1* gene, we can estimate the lengths of the inserted segments, designated *a* and *b*, to be 36 and 57 base pairs, respectively. Subsequent to heteroduplex formation, these segments are copied independently into the adjacent shorter heteroallele. In the heteroduplex formed between the S and F parental alleles, gene conversion is initiated, perhaps by a single strand break in the (short) strand opposite insertion *a* or *b*, and completed by gap formation and gap-filling repair replication.

Although the two insertions are rather similar in size, our data clearly show that the efficiency of execution (probably of the initial step of the gene conversion event) is much greater for segment *a* than for segment *b*. Not only is IF the most abundant non-parental form of *var1* polypeptide issued in the original F×S cross, but also in subsequent backcrosses, in all pairwise matings which could yield a heteroduplex between a strand carrying insertion *a* and one lacking it, the latter is frequently converted to a form containing the *a* insertion. As shown in

Figure 5, this may be seen either as the generation of a non-parental form by conversion of one of the parental alleles (such as in the cross IF×IS) or as an unequal transmission of the parental forms (as in the crosses between IF×F and IS×S). On the other hand, backcrosses giving rise to heteroduplex pairs where both strands either contain or lack insertion *a*, although heteroallelic or homoallelic for insertion *b*, show little change in the output of the parental forms. This result is entirely consistent with the observation that the formation of IS, which formally represents the insertion of segment *b* into F in the S×F cross, occurs at a frequency of less than 2%.

The model presented here bears a similarity to the events suggested to occur at the ω locus in heteropolar ($\omega^+ \times \omega^-$) crosses^{4,9}. Gene conversion at ω was proposed to be a fundamental feature of mitochondrial transmission genetics^{7,8}. It was further suggested that gene conversion might be a general feature of recombination in yeast mtDNA. As is the case at the ω locus, this could be asymmetric, giving rise to a gradient in transmission of markers and grossly unequal numbers of reciprocal recombinant genotypes, or symmetric, in which case coordinate transmission of markers and equal numbers of reciprocal recombinant types would be obtained. Since that time, the analysis of recombination in other regions of the genome failed to reveal additional examples of efficient polar recombination^{19,20} so that the events at ω were then viewed as an interesting but special case. We are prompted by our present results to reappraise the relative role of gene conversion as an important feature of the inheritance of mitochondrial genes.

The high frequency of the recombination (gene conversion) events at *var1* described here and those at other regions of the mitochondrial genome, may be explained as a consequence of multiple rounds of recombination in each cell generation⁴. Although that is almost certainly an important property of yeast mitochondrial genetics, it is possible that site-specific recombination may also be involved. In particular, insertions or base sequences at the ends of insertions, may play an important part

in mitochondrial recombination. It will be of great interest to learn whether any of these insertions have structural features or base sequences in common and to extend the analysis of the mode of inheritance of other insertions present in mtDNA from various yeast strains to those both larger and smaller than ω^+ .

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Mycoplasma capping on lymphocytes

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When mycoplasmas infect lymphocytes they behave as multivalent ligands and cap on the lymphoid cell surface in the absence of added specific antibody. There is an apparent high correlation between mycoplasma capping and blast transformation of the infected lymphocytes. Mycoplasma caps are shed from the surface of cells as an aggregate containing host membrane vesicles. This novel interaction may suggest a physiological role for the phenomenon of capping and may play a part in mycoplasma pathogenesis.

MYCOPLASMAS are extracellular parasites often found in close association with the host cell plasma membrane^{1,2}. Their predilection for cell surfaces, especially moist mucosa, is responsible in part for their involvement in pathological states such as chronic respiratory disease, polyarthritis and genital tract infections in many species of animals. Little is known about the specific nature of mycoplasma–host cell interactions either *in vivo*, where the parasites have a fairly strict host species specificity, or *in vitro*. Although specific cell receptors have been described for a few mycoplasma species^{3–5}, their chemical identities for the most part remain obscure.

We have investigated the nature of these interactions *in vitro* and their possible relevance to the pathogenic consequences of mycoplasma infection^{6,7}. We describe here a novel interaction between mycoplasmas and lymphocytes *in vitro*, in which the mycoplasmas behave as multivalent ligands and cap on the surface of infected lymphocytes in the absence of specific antibody.

Mycoplasma infection of an established lymphoblastoid line

Using a strain of *Mycoplasma hyorhinis* adapted to rapid cell colonisation, we infected a mouse lymphoma line, S49 (ref. 8), with mycoplasmas at multiplicities of infection of 1–10 viable organisms per cell. Infected cells were grown in suspension in RPMI medium containing 10% calf serum at 37°C and then processed for electron microscopy and immunofluorescence 1–2 d after infection.

Viable control and infected cells were stained with fluorescein-conjugated anti-*M. hyorhinis* antiserum diluted in isotonic buffered saline. To compare the distribution of mycoplasmas on the surface of lymphocytes as a function of the lateral mobility of their receptors in the fluid membranes^{9–11}, antibody staining was carried out at room temperature and 0°C, and also on lymphocytes poisoned with 5×10^{-3} M sodium azide just before staining. To establish the rate of appearance of caps, S49 cells were stained with fluorescein-conjugated anti-mycoplasma antibody at intervals during a 24-h period after infection with *M. hyorhinis*.

Cells were also fixed in glutaraldehyde and then post-fixed in osmium tetroxide. After dehydration and embedding in resin, thin sections were cut, stained and examined in a Philips 300 electron microscope.

Mycoplasma infection of mouse spleen cells

Spleens were removed from 6–8-week-old BALB/c and *nu/nu* (nude) athymic mice, and the spleen cells suspended in RPMI medium containing 10% calf serum. After treatment with ammonium chloride to lyse erythrocytes, the spleen cells were seeded at approximately 5×10^6 per ml in RPMI medium containing 10% calf serum and 5×10^{-5} M 2-mercaptoethanol.

Table 1 Rate of mycoplasma capping on S49 cells

Time after mycoplasma infection (h)	% Cells infected		% Cells capped		% Cells patched	
	PBS	5×10^{-3} M NaN ₃	PBS	5×10^{-3} M NaN ₃	PBS	5×10^{-3} M NaN ₃
1	0.65	0.58	0.0	0.01	0.65	0.57
4	6.40	6.62	2.80	2.65	3.60	3.97
8	17.66	21.20	7.85	8.40	9.81	12.80
16	22.60	22.57	19.20	18.50	3.40	4.07
24	43.35	40.46	38.23	35.80	5.12	4.66

S49 lymphoblastoid cells (3×10^6 cells ml⁻¹) were infected with *M. hyorhinis* at 5×10^7 colony-forming units per ml. At the indicated time intervals cells were washed with phosphate-buffered saline (PBS), stained with fluoresceinated anti-*M. hyorhinis* antiserum diluted in PBS, and mounted in 1:1 PBS:glycerol. The preparations were then scored for cells exhibiting specific fluorescence. At least 1,000 cells were scored for each determination. Cells treated with 5×10^{-3} M sodium azide (NaN₃) were exposed to this inhibitor during the entire process of washing, staining and mounting. In addition, these azide-treated cells were processed at 0°C. Untreated cells were processed identically except that NaN₃ was omitted from the reagents and the manipulations were carried out at room temperature.

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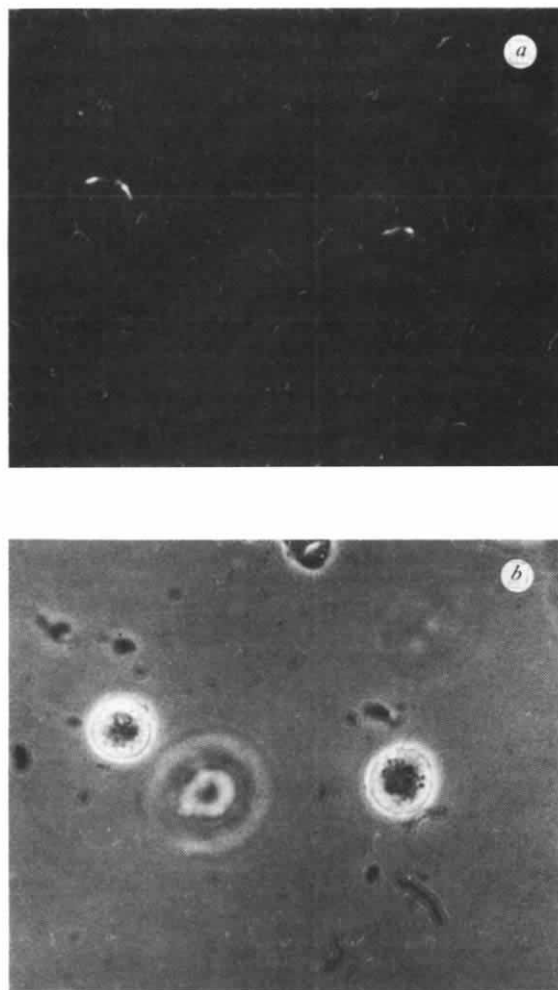


Fig. 1 S49 lymphoblastoid cells infected with *M. hyorhinis*. *a*, Capped mycoplasmas revealed with fluoresceinated anti-mycoplasma antiserum 24 h after infection. Magnification, $\times 1,200$. *b*, Phase microscopy of the same field. Magnification, $\times 1,200$.

Cells were then infected with $1-5 \times 10^7$ colony-forming units of *M. hyorhinis* and incubated at 37°C . Cultures were taken daily for 3 d and blast formation was scored using phase optics according to the number of blast cells in a given lymphocyte population.

The number of dead cells was estimated by staining the unfixed populations with $0.5 \mu\text{g ml}^{-1}$ 4'-6-diamidine-2-phenylindole (DAPI), which enters dead cells and binds specifically to DNA. Thus, the nuclei of dead cells fluoresced when examined microscopically using the Leitz Ploem system whereas those of live cells did not. The degree of blast transformation was confirmed by fixing cells with 0.5% glutaraldehyde on ice for 10 min followed by staining with Jenner-Giemsa stain. Cap formation was monitored by immunofluorescent staining as described above. For comparison, parallel cultures of splenic lymphocytes were treated with $10 \mu\text{g ml}^{-1}$ of bacterial lipopolysaccharide (LPS), a potent mitogen for B lymphocytes¹².

We did not attempt to measure lymphocyte stimulation by the conventional thymidine incorporation assay¹⁶ because our preliminary experiments (unpublished) gave highly variable results due to the nucleoside phosphorylase activity of mycoplasmas which catalyses the rapid conversion of thymidine to thymine^{17,18}.

Mycoplasma capping on S49 cells

The rate of formation of mycoplasma caps on the S49 lymphoblastoid cells is shown in Table 1. Initially, fluorescence was seen in very few cells and was predominantly distributed in an

irregular patched pattern over the cell surface. Several hours after infection, the frequency of caps increased (Fig. 1), reaching a peak approximately 24 h after infection. There was no significant difference in the frequency of mycoplasma caps in the presence or absence of 5×10^{-3} M sodium azide, which is an inhibitor of respiration and consequently of energy-dependent capping¹¹. This indicates that the mycoplasmas were behaving as multivalent ligands independent of specific anti-mycoplasma antibody. The kinetics of appearance of the caps after being distributed over the entire cell surface, as evidenced by early ring and patch fluorescence followed by capping, indicated a process of interaction and movement similar to that seen with ligands such as concanavalin A (refs 13, 14), albeit slower in rate.

Capping in the absence of specific antibody was clearly established in our ultrastructural studies. As Fig. 2 shows, mycoplasmas were commonly situated at one pole of a cell when examined by transmission electron microscopy. Scanning electron microscopy (Fig. 3) showed the polar accumulation of capped mycoplasmas particularly distinctly. Accumulation was often sufficient to deform the lymphocytes physically, and was also seen in the phase and immunofluorescent preparations shown in Fig. 1. Uropods formed at the sites of mycoplasma accumulation (Fig. 4), and the mycoplasmas clustered around the uropod were often associated with small membranous vesicles, presumably derived from the host cell surface membrane. Towards the latter stages of the 24-h infection period the mycoplasmas were shed from the surface of the lymphocytes as large aggregates containing the membranous vesicles of

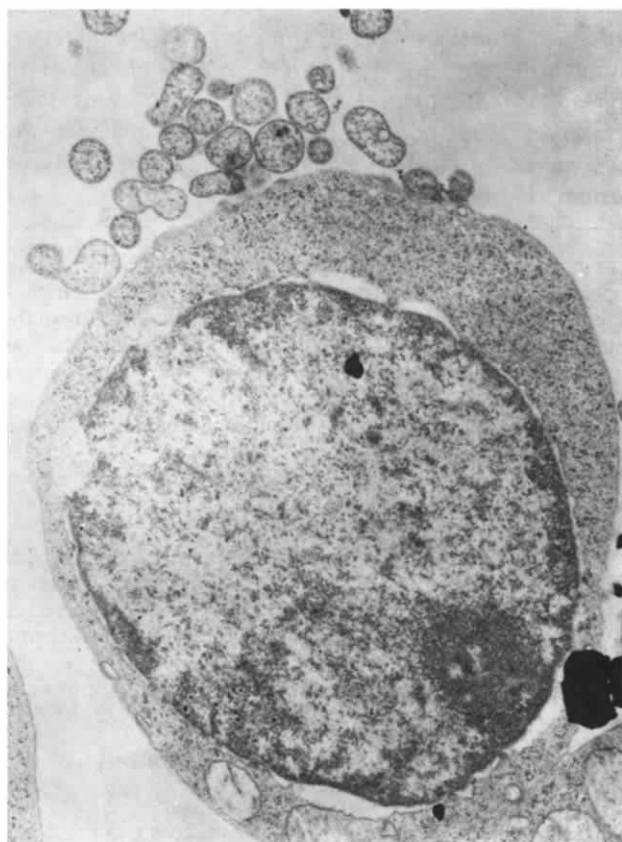


Fig. 2 S49 lymphoblastoid cell infected with *M. hyorhinis*. A cap of mycoplasmas is located at one pole of the cell. Cells were chilled on ice, washed once in 0.2 M Na cacodylate buffer, pH 7.2, fixed in the same buffer continuing 3% glutaraldehyde for 1 h, pelleted and fixed directly in 1% osmium tetroxide in Veronal buffer (pH 7.2; 0.5 mM CaCl_2). The pellet was then dehydrated in ethanol. Fixed cells in 100% ethanol were then warmed to room temperature, infiltrated with resin and further processed as described in the text. Magnification, $\times 18,000$.

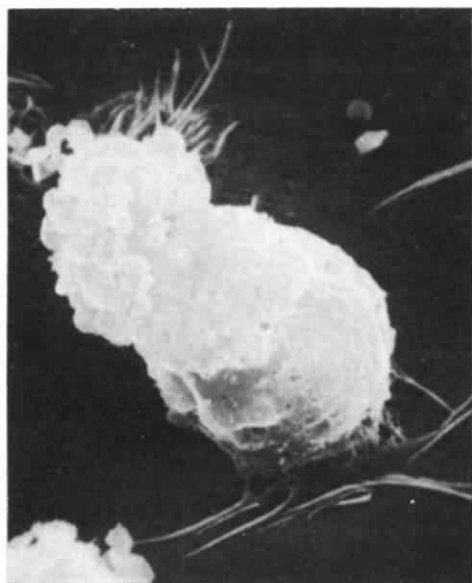


Fig. 3 Scanning electron micrograph of an S49 lymphoid cell with a prominent cap of mycoplasmas. Fixation was accomplished by diluting with 0.2 M potassium phosphate buffer containing 2.5% glutaraldehyde, pH 7.5, 1:1 with medium, at room temperature. Cells maintained their original shapes at this dilution. The cells were washed with 0.1 M potassium phosphate buffer, pH 7.4, and allowed to attach to the surface of coverslips on which a thin layer of 1% poly-L-lysine (MW 70,000) had been dried³⁴. Dehydration in ethanol was followed by transfer to acetone and critical point drying. Magnification, $\times 7,900$.

presumed host origin. We saw no evidence of endocytosis, which is often seen when surface antigens are capped by antibodies^{14,15}. In immunofluorescent preparations, shed caps appeared as distinct, freely floating, highly fluorescent aggregates.

Blast transformation of mouse spleen cells

M. hyorhinis behaved as a potent mitogen for both BALB/c and *nu/nu* splenic lymphocytes. Table 2 shows that the mycoplasmas were more effective than LPS in stimulating blast transformation. Although blast cells were not scored as such until obvious morphological changes had taken place, we noted subjectively that 'preblasting' (that is, the appearance of medium lymphocytes) occurred earlier and involved more cells in the mycoplasma-infected cultures than in the LPS-treated cultures, particularly those derived from *nu/nu* spleens.

Examination of infected cells by immunofluorescence revealed a series of events (Table 3) similar to that observed in S49 cells. Within 24 h of infection, approximately 40% of cells showed evidence of infection as measured by specific fluorescence. Most of these cells had distinct fluorescing caps and the remainder exhibited patchy fluorescence. Relatively few blast cells were visible but virtually all were capped. A significant number of blast cells had small pinpoints of specific fluorescence at a single pole of each respective cell, especially in the *nu/nu* cultures. By the second day of infection the number of blast cells with polar pinpoints of infection had increased considerably. In *nu/nu* spleen cultures in particular, many blast cells were seen which had no membrane-associated fluorescence; by day 3 most *nu/nu* blast cells failed to exhibit specific fluorescence.

We consider these series of observations to indicate that the process of infection, patching and capping is closely related to the induction of blast transformation in these cells. Furthermore, the caps seemed to be shed from the surface of the blasts, and reinfection of the stripped cells did not recur during the time of observation. Very few of the unstimulated or dead cells on days 2 or 3 were infected, as measured by specific immunofluorescence.

Significance of mycoplasma capping

The phenomenon of capping, first described by Taylor *et al.*¹¹, convincingly demonstrated that cell surface receptors which are normally distributed randomly in the plane of the membrane may be redistributed into patches and caps after crosslinking by multivalent ligands. This original study and subsequent reports^{14,15,19} demonstrated capping by crosslinking surface antigens with antibodies directed against the surface antigens or by using soluble ligands such as concanavalin A (refs 13, 20). We believe that the studies described here are the first report of an infectious organism behaving in a fashion similar to soluble multivalent ligands and of capping on the surface of lymphocytes in the absence of specific antibody. Certain viruses are distributed in patches on the surface of lymphocytes in the absence of antibody, but the addition of specific antibody is required to induce capping²¹.

The association between capping and blast transformation is also particularly striking. We do not yet know if mycoplasma capping is causally related to lymphocyte stimulation, but if this proves to be the case it may portend a physiological significance for capping during microbial infection. Several mycoplasma species have been shown to induce blast transformation of splenic lymphocytes *in vitro*²²⁻²⁵ and result in polyclonal B-cell activation²⁶. It will be interesting to see if there is a general

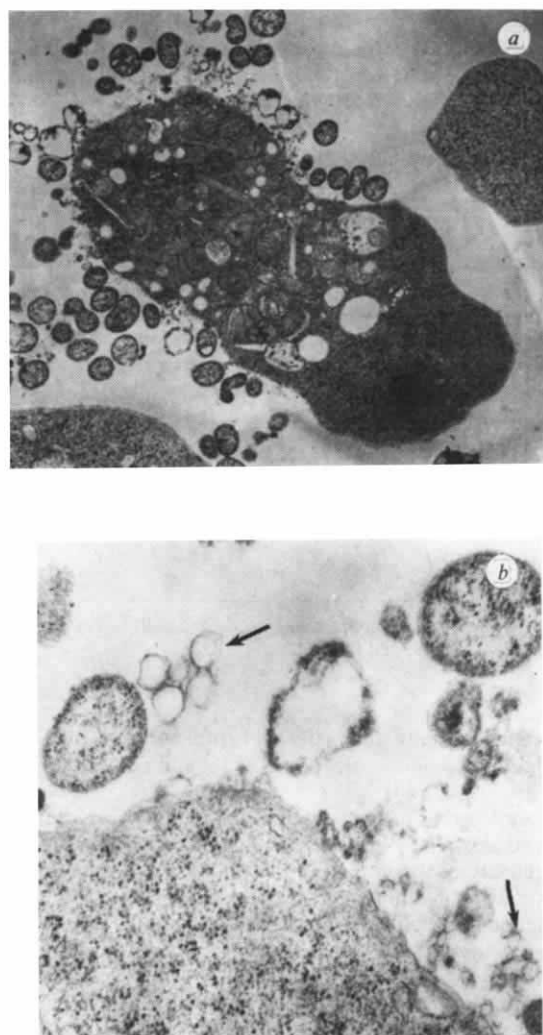


Fig. 4 Mycoplasma caps on S49 lymphoblastoid cells. *a*, Thin section of lymphocyte containing a broad cap of mycoplasmas associated with a uropod. Magnification, $\times 8,900$. *b*, Higher magnification of a uropod showing membrane vesicles (arrowed) released from the cell surface. Magnification, $\times 40,000$.

Table 2 Comparative mitogenic effects of bacterial lipopolysaccharide and mycoplasmas on mouse lymphocytes

Time post-treatment or infection (d)	Treatment of cells	BALB/c splenic lymphocytes			nu/nu splenic lymphocytes		
		Total cells counted	% Blast cells	% Dead cells	Total cells counted	% Blast cells	% Dead cells
1	Control	483	0.6	24.6	503	1.3	24.4
	LPS	509	1.9	25.5	503	8.1	22.0
	<i>M. hyorhina</i>	459	4.3	26.3	444	9.2	26.8
2	Control	446	0.4	31.1	380	1.5	36.2
	LPS	402	15.1	23.8	350	22.7	22.4
	<i>M. hyorhina</i>	429	37.0	34.9	362	38.3	36.7
3	Control	350	0.6	51.8	351	7.1	36.3
	LPS	367	27.5	23.6	355	29.5	23.3
	<i>M. hyorhina</i>	356	41.2	49.2	384	52.6	41.6

The blastogenic transformation of the splenic lymphocytes was scored using phase optics and confirmed following fixation and staining with Jenner-Giemsa. Dead cells were determined by DAPI staining as described in the text. Blast cell percentages were calculated as per cent of total cells.

correlation between mycoplasma capping and lymphocyte activation with these other species. The strong correlation between mycoplasma caps and blast transformation suggests that this may well be the case.

Our preliminary results presented here also indicate that mycoplasmas seem to be a more potent mitogen than LPS. The isolation of the active mycoplasma components will clarify this possibility. The fact that both BALB/c and athymic nude splenic lymphocytes were stimulated suggests that the predominant cells affected are B lymphocytes. We have confirmed this using anti-immunoglobulin sera but have also found that a proportion of the stimulated cells do not bear immunoglobulin (Ig) on their surface (unpublished observations). We do not yet know if these represent a population other than B cells or whether surface Ig has been co-capped with the mycoplasmas. Our preliminary

An alternative way in which mycoplasma-host cell interactions may lead to autoimmune reactivity is by antigenic modulation. It is well established that during infection certain viruses alter the composition of the host cell's plasma membrane^{31,32}. The changes which ensue include particularly the depletion or loss of histocompatibility antigens from the surface³². Antigenic modulation of cells following viral infection has been linked to the immunopathology caused by these infections. We have preliminary evidence that infection of mouse lymphocytes with mycoplasmas leads to the depletion of host cell surface antigens, including H-2 (unpublished observations). Similar losses of host antigens following mycoplasma infection of lymphocytes have been noted by other investigators³². It is possible that these surface antigens may serve as mycoplasma receptors and are stripped from the cell during the

Table 3 Relationship between mycoplasma capping and blast transformation

Time after mycoplasma infection (d)	BALB/c splenic lymphocytes							nu/nu splenic lymphocytes						
				Blast cells	% Blast cells infected						Blast cells	% Blast cells infected		
	% Cells infected		Total		Capped	Patched	% Cells infected		Total	Capped		Patched		
	Total	Capped					Patched	Total					Capped	Patched
1	41.4	33.6 (24.8)	7.8	8.8	89.4	52.6 (15.7)	36.8	32.4	24.8 (20.4)	7.6	14.7	83.7	61.1 (44.4)	22.6
2	60.0	30.2 (18.6)	29.8	49.2	100	62.5 (33.4)	37.5	25.6	22.0 (20.7)	3.6	47.3	25.2	24.0 (24.0)	1.2
3	29.8	24.1 (19.3)	5.7	51.1	46.8	44.5 (30.2)	2.3	15.5	13.7 (12.6)	1.8	58.0	14.0	13.2 (12.6)	0.8

The distribution of mycoplasmas on infected cells was determined by specific immunofluorescence. Percentages in parentheses refer to pinpoint polar capping as discussed in the text.

results with partially purified T cells indicate that they too are stimulated to a lesser degree (E.J.S. and B. Pires, in preparation).

Certain features of the mycoplasma-lymphocyte interactions described above may be important for our understanding of the pathogenicity of these microorganisms. Mycoplasma infections are usually self limiting but are occasionally followed by complications which are autoimmune in nature². For example, *M. pneumoniae* causes primary atypical pneumonia in humans, a condition which is usually limited to the respiratory tract. However, documented sequelae to this infection include anaemia and neurological complications^{2,27,28}. Also, antibodies directed against host tissue, including the I antigen of erythrocytes, lung and brain, have been found in the sera of patients infected with *M. pneumoniae*^{28,30}. If mycoplasmas are capable of inducing B-cell activation *in vivo*, as they are *in vitro*, it is possible that 'forbidden' clones may be activated to produce autoantibodies.

capping process and subsequent release of the cap from the surface of the lymphocyte.

In conclusion, we have shown that a novel feature of the infection of mouse lymphocytes with mycoplasmas is the capping of these organisms at one pole of the cell. Mycoplasma capping is correlated with a high efficiency of blast transformation; thus, mycoplasmas behave as potent mitogens. The caps are shed as aggregates from the surface of the lymphocytes and contain membranous vesicles which are presumably of host origin and may contain mycoplasma receptors. If this interaction has a parallel *in vivo* it may explain some of the autoimmune sequelae to mycoplasma infection.

We feel it is also appropriate to include a final note of warning that investigators using lymphocytes as model systems to investigate mitogenicity, lymphocyte triggering, surface antigens and viral pathology should, together with all other investigators using cultured cells, beware of mycoplasma contamination^{1,7}.

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letters

HEAO Observations of X-ray bursts from MXB1730–335

BURST activity was observed¹ on 18 March 1978, from MXB1730–335, the rapid burster, from which no activity had been observed a few days earlier². HEAO 1 was pointed (+y axis) at the rapid burster from ~18.00–21.00 UT on 31 March 1978. The source was actively bursting in the 'Mode I' pattern³, in which very large type II bursts⁴ are first followed by a long (several minutes) quiescent period and then by a train of closely spaced, small bursts. Because type I and type II bursts probably have different underlying physical mechanisms⁴, it is important to study both types over a large energy range. We report here on a search for hard X-ray emission from the rapid burster and on evidence for spectral evolution in the type II bursts.

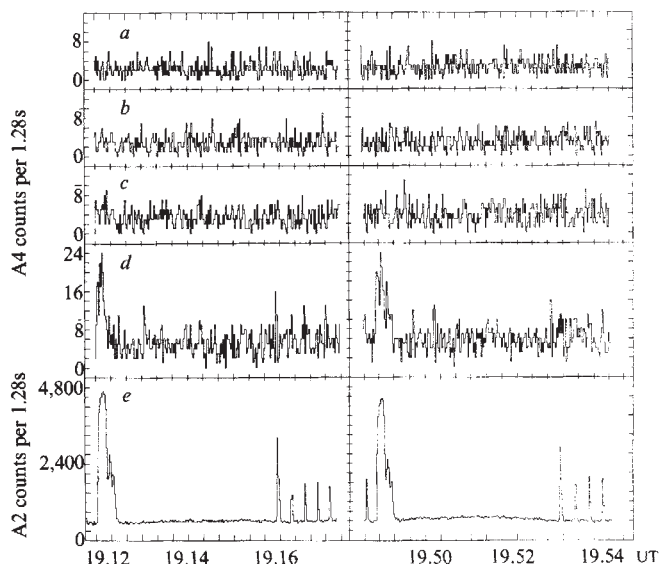
Fifty-eight minutes of quick-look data were examined from the A2 experiment and from the A4 experiment on HEAO 1. Two very large bursts were observed in both the A2 and A4 data (Fig. 1). Each lasted ~25 s and had a total energy flux (2–60 keV) of $3\text{--}4 \times 10^{-7}$ erg cm⁻². Five moderately sized bursts (~10 s duration, $\sim 10^7$ erg cm⁻²) and 167 small bursts (~5 s duration, $2\text{--}5 \times 10^{-8}$ erg cm⁻²) were also observed in the A2 data. The two large bursts which occurred at 19 h 12 min 00 s UT and 19 h 48 min 54 s UT, are shown in Fig. 1, as observed in four energy channels from 13–160 keV by the A4 low energy detectors⁵ (sum of count rates in two independent detectors each with an area of ~100 cm²) and in a single, broad energy band, by the A2 detectors. The A2 data shown here are the sum of various energy channels in three separate detectors⁶, spanning the energy range 2–60 keV. The A4 data were binned on-board the spacecraft into 0.64 s time bins and ~5 keV energy bins. Background counting rates were determined from ~200 s of data immediately following the 19 h 12 min 00 s burst and from ~35 s of data following a moderate-size burst ~13 min later (not shown in Fig. 1). They include the diffuse X-ray and charged particle background and any steady emission that might come from the rapid burster or from 4U1728–33 which are separated by only ~0.5°.

Because of the steep spectrum of the type II bursts from the rapid burster³, more than 95% of the total A2 counts is due to photons with energies <13 keV. These data can therefore be

considered effectively as an energy channel (2–~13 keV) preceding the first A4 channel (13–25 keV). The large type II bursts from MXB1730–335 in the A2 data seem to be similar to those observed in 1976 (refs 7–9), showing a broad maximum and a double-peaked structure during the burst decay.

Each burst was analysed in two nearly equal time segments. The ratio of counts in the last to the first part of the 19 h 12 min 00 s burst was 0.353 ± 0.004 in the A2 data and <0.15 (3 σ confidence level) in the 13–25 keV channel of the A4 data. No significant detection of X rays was made at all in the A4 data after the first 13 s of the burst, while in the A2 data, at lower energies, the burst lasted ~25 s. The ratio of counts in the

Fig. 1 HEAO 1 data for the rapid burster (MXB1730–335) on 31 March 1978. The enhancement after the large bursts is probably 'steady' X-ray emission as previously observed from the rapid burster^{4,19}. a, 85–160 keV; b, 40–85 keV; c, 25–40 keV; d, 13–25 keV; e, 2–60 keV (95% of counts <13 keV).



last to the first half of the 19 h 48 min 54 s burst was 0.467 ± 0.006 in the A2 data and 0.30 ± 0.08 in the A4 13–25 keV channel. In both bursts, the X rays with energies above 13 keV decayed faster than the lower energy X rays; that is, the spectra softened during the burst decay. The spectral evolution of the bursts can perhaps also be seen from the A2 data alone. In both xenon detectors in which the bursts were observed, the ratios (comparison of second and first half of the bursts) for counts above 9 keV were less than the ratios for counts below 7 keV, with a significance slightly greater than 3 s.d.

We conclude that the type II bursts from the rapid burster do show a spectral softening during burst decay. However, this spectral softening is not nearly as pronounced as in type I bursts, either from MXB1730–335 (ref. 4) or from other burst sources^{10–13}. Marshall *et al.*³ found that the spectra of type II bursts from MXB1730–335 as measured by SAS 3 do not change significantly during burst decay. However, the SAS 3 data were not sensitive to the spectral evolution reported here. The highest energy channel with a statistically significant signal in the SAS 3 data was 8–19 keV. For the spectrum of these bursts, this energy channel is dominated by X rays below 10 keV, and the spectral softening reported here could not have been detected.

We searched for the existence of a flux in the 86–160 keV energy range. Combining all the data (corrected for collimator transmission), we find a 90% confidence upper limit¹⁴ of $<1.7 \times 10^{-8}$ erg cm⁻² per burst in the range 85–160 keV corresponding to $<2.0 \times 10^{38}$ erg per burst at a source distance of 10 kpc. The average energy of these two bursts as detected by A2 in the 2–20 keV energy range was $(4.4 \pm 0.5) \times 10^{39}$ erg per burst at 10 kpc. This value is similar to that measured previously for large bursts from the rapid burster^{7,8}. Thus large type II bursts from the rapid burster contain $<5\%$ of their total X-ray energy in the range 85–160 keV.

This lack of a hard X-ray flux has consequences for those accretion instability models of X-ray bursts which require extremely high temperatures; for example, super-massive black holes ($\sim 10^5 M_\odot$) surrounded by a $\sim 10^9$ K hot cloud¹⁵ or spherical accretion of compact H II regions on less massive black holes (~ 10 – $100 M_\odot$)¹⁶. In the latter, the low energy (<20 keV) burst should be accompanied by at least as luminous a burst in hard X rays (≥ 100 – 200 keV). Our present observations, of the absence of any appreciable flux in the range 85–160 keV, do not, of course, rule out the possibility that a large amount of burst energy is present above 160 keV.

Other evidence has accumulated that sources of type I bursts are not massive black holes but objects of solar mass^{9,13,17,18}. As the rapid burster itself is also a source of type I bursts⁴, these results for the type II bursts (which are almost certainly due to accretion instabilities) then limit the production of hard X rays that are allowed in the accretion on a neutron star or a small black hole.

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NGC326—a radio galaxy with a precessing beam?

THE radio emission from most strong extragalactic sources is concentrated in a band which passes through, and is symmetrical with respect to, the associated galaxy¹. This structure is sometimes modified to a V or C shape with the galaxy at the apex, as in the wide angle tail sources like 3C465 (ref. 2), and occasionally to the more extreme elongation of the tail sources such as NGC1265 (ref. 3). In a few sources an S-shaped or 180° symmetry is seen. This was first noted in the outer components of Cen A⁴ and a better example is 3C272.1 (refs 5, 6). Other sources where the S distortion is very clear have been found with higher resolution observations (for example Cyg A⁷ and 3C47⁸). In these, the structure in question subtends a small angle from the centre and they give a strong impression of a beam type of model in which the beam has wobbled or precessed by a few degrees⁹. A new example of a radio source showing a structure with a 180° symmetry, but not of the S form, has been found as part of a systematic study of radio galaxies of lower luminosity selected from the B2 catalogue¹⁰. The projection of a precessing beam model seems to provide a natural explanation for this strange shape and is described here.

The radio source B20055+26 (426.03) was first mapped with the Westerbork Synthesis Radio Telescope in a program at 1.4 GHz in which short observations were obtained of all the B2 radio sources identified with bright galaxies¹¹. Because an interesting structure was seen this was followed up by a 2×12 h observation at 5 GHz resulting in a map with resolution $6'' \times 13''$ (RA $\times$ dec) and r.m.s. noise of 0.8 mJy (1 mJy = 10^{-29} W m⁻² Hz⁻¹) beam area. This map is shown in Fig. 1 and the position of the elliptical galaxy NGC326 is indicated. This galaxy is the brightest member of a small group of galaxies, Zwicky 0056.9+2636, and has a redshift of 0.0472 (ref. 10). There is a fainter elliptical galaxy 2' NE and the other members of the group are more than 5' away.

The radio map shows two curving tails with a 180° symmetry. There is also emission at the position of the galaxy. This may be a

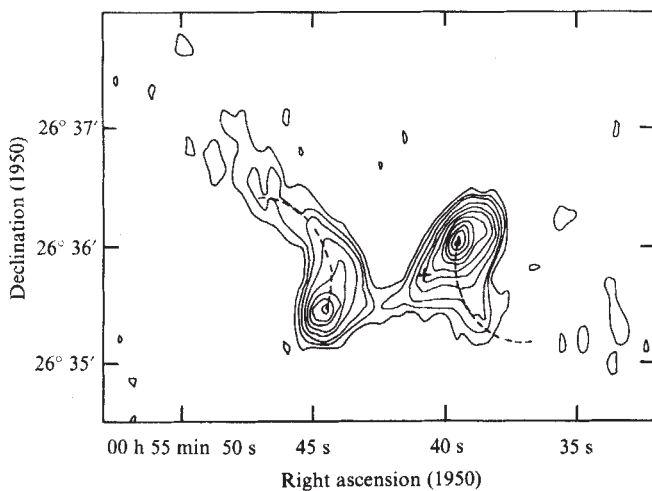


Fig. 1 5.0 GHz map of the brightness distribution of B2 0055 + 26. Contours are 2, 4, 6, 8, 10, 15, 20, mJy per beam area. The r.m.s. noise level is 0.8 mJy per beam area. 1 mJy per beam area = 0.53 K. The position of the galaxy NGC326 is indicated by the +. The dashed lines shown the trajectories of the ends of the beams for the model discussed in the text.

separate compact source (such as is often seen in radio galaxies at this frequency) which is blended together with the emission from the western component or it may be part of a bridge connecting the galaxy and the heads of the two components. The 180° symmetry is even more striking if the emission at the position of the galaxy is a separate compact source because then the ridge of extended emission will curve south from the west component in the identical way that the tail curves to the north-east from the eastern component making the symmetry almost perfect about a position RA = 00 h 55 min 42 s, dec = +26°35'44", 0.5' east of the galaxy.

Comparison of the 5 GHz map with the 1.4 GHz data indicates a spectral index of -0.7 in the bright inner regions and this steepens to a value of ≈ -1.1 in the tail going to the north-east and in the very low brightness region just visible above the noise in the south-west corner of Fig. 1. Other data for this radio galaxy are summarised in the Table 1.

Either component taken separately has a structure remarkably similar to that seen in the tail radio sources but the morphology of the two components together and the location of the galaxy make it impossible to ascribe their shape to a trajectory of the galaxy in this case.

Complex radio source morphologies are now often attributed to the interaction with the intracluster medium, for example, Lari and Perola¹² have shown that in a given luminosity range the number of complex sources found inside rich clusters of galaxies is considerably higher than the number found outside.

Table 1 Observational Data for NGC326—B2 0055 + 26

Red shift ¹¹	0.0472
m_{pg}^{11}	14.9
Optical position ¹¹ (1950)	RA = 00 h 55 min 40.7 s dec = +26°35'44"
Total flux density:	
0.4 GHz	4.9 Jy
0.6 GHz	3.15 Jy
1.4 GHz	1.7 Jy
5.0 GHz	0.69 Jy
Angular size	4'
Distance*	141 Mpc
Linear size	160 kpc
Radio power (1.4 GHz)	$3 \times 10^{23} \text{ W Hz}^{-1} \text{ ster}^{-1}$

* $H = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

This radio source is not located in a rich cluster and, furthermore, its distortion has a symmetry which cannot readily be explained as an effect of the surrounding medium. Thus we are led to conclude that in this case the shape is determined by the source energising the radiating plasma rather than by the surrounding medium. The observation of the change in spectral index implies that the regions of high brightness are the youngest so we might consider models in which the energy is supplied by a beam which has rotated through about 90° while generating the source and now points to the region of high brightness. At first sight this kind of model seems somewhat implausible as the region just behind the head is closer to the galaxy so requiring a variation in the power of the beam which is not reflected in the monotonic change of brightness. However, when we consider the effect of projection on the trajectories of the ends of a pair of beams sweeping out two cones on either side of the galaxy (Fig. 2) we can find an impressive agreement with the observations. The dotted lines in Fig. 1 show the trajectories of the ends of the beams in a model in which the beam is offset from the axis of rotation by 18° and with the axis of rotation inclined to the line of sight by 30°. In such a model the observed change in brightness from the head to the end of the tail is a natural consequence of the increasing age.

Models in which the radiating plasma is energised by a beam emanating from the nucleus of the galaxy have been proposed by Rees¹³, Longair *et al.*¹⁴ and Blandford and Rees¹⁵. Furthermore, Miley⁹ and Hendrikson and Bridle (unpublished) have suggested that the S symmetry seen in some sources could be due to the changing orientation of such a beam. For NGC326 the high degree of symmetry and excellent fit to the model indicates a fairly uniform rotation of a beam offset from the rotation axis by a relatively large angle and generating the radiating region at a constant distance from the galaxy. The continual motion of the beam through the intergalactic medium and the maintenance of a constant distance from the galaxy to the radiating region will present some interesting problems and constraints on the types of beam models proposed¹³⁻¹⁵.

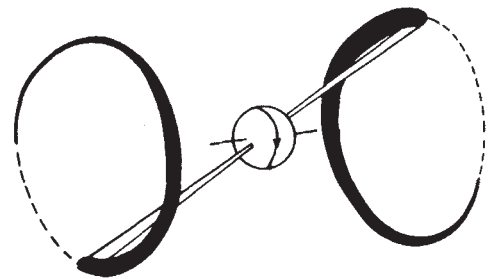


Fig. 2 A sketch showing the essential features of the proposed model.

The rotating offset beam may be caused by a real offset between the beam and the rotation axis of the material supporting it, or the beam may be aligned with the rotation axis of a gaseous disk in the galaxy which is precessing because of a difference between its rotation axis and the principal axis of the galaxy as a whole. The observation of a difference between the rotation axis of the H I and the optical minor axis of the elliptical galaxies NGC4278 (ref. 16) and NGC1052 (ref. 17) provides support for the latter speculation.

We can make a rough estimate of the time scales involved from the observed steepening of the radio spectrum. If this steepening is due to energy losses the age of the ends of the tails would be $\approx 4 \times 10^7$ yr, however, in tailed radio sources we know that the ages estimated from the kinematics are considerably longer than those determined from the spectral steepening^{18,19}. If we compare the steepening of the spectrum seen in this source with that seen in tail sources then the age of the end of the tail is more likely to be $\approx 2 \times 10^8$ yr corresponding to a period of

$\sim 6 \times 10^8$ yr for the full circle. In the inner part of elliptical galaxies where solid body rotation is seen the rotation periods are about 10^7 yr (ref. 20), which may be too short to explain the radio data on the basis of the offset beam. If the motion of the beam is caused by precession of a gaseous disk the longer time scale implied by the radio data becomes reasonable.

If the radio axis is associated with a much smaller object such as a black hole in a tilted accretion disk then the motion of the axis will be related to the Bardeen and Petterson effect²¹ as discussed by Rees²².

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Limits on cosmic radio bursts with microsecond time scales

IT has been suggested¹ that black holes of mass $\leq 10^{15}$ g evaporate in $\sim 10^{10}$ yr, ultimately annihilating into a burst of energetic photons and particles. This explosion would produce γ -rays directly, and a radio pulse with a characteristic frequency of ~ 3 GHz and an energy of $\sim 10^{32}$ erg may also be generated². It has been shown²⁻⁴ that such a radio burst would be far more easily detected than the corresponding γ -ray burst. Estimates of the energy in the radio burst are uncertain by many orders of magnitude, and it is, of course, not known whether any primordial black holes exist at all; however, the implications of a detection would be enormous for quantum and gravitational physics.

The observational requirements to make an optimal search for a pulse with a ~ 3 GHz frequency are in themselves interesting as the predicted pulse is only one radio frequency cycle in duration and by definition this event will never be repeated. Such a short pulse will contain all radio frequencies up to a cut-off determined by the actual duration of the pulse so an optimum receiver would have very wide bandwidth, B , and a time constant limited only by the reciprocal of this bandwidth. Unfortunately any pulse originating outside the local region of the galaxy will be dispersed by the interstellar medium thereby reducing the maximum possible sensitivity. For example, in a

10 MHz band at 5 GHz we would expect dispersion to smear pulses to ≥ 10 μ s. It is interesting to note that pulses of this character would not be detected with the time constants commonly used in radio astronomy (even for pulsar surveys) although such a pulse can easily be detected with a suitably designed receiver. The existence of this unexplored time domain for astrophysical phenomena provides additional incentive for such a search.

Limits on the radio pulse emission rate from exploding black holes have already been set by using negative results from wide-beam searches for radio pulses such as might be produced by supernovae^{3,4}. In contrast to the small, low-gain antennas used in those searches, we have used the 25-m Dwingeloo radiotelescope; our objective was not only to probe large volumes of space for possible bursts from exploding black holes, but also to observe a variety of specific objects with high sensitivity and time resolution.

Table 1 Sources observed

	Source	Time on-source (h)	Limiting energy (erg)
Planet	Jupiter	2	6×10^6
Star	Algol	3	1×10^{25}
Pulsars	PSR0950+08	3	3×10^{26}
	PSR1133+16	3	3×10^{27}
Supernova remnant	Crab	1	2×10^{30}
X-ray sources	Sco X1	2	3×10^{29}
	Her X1	1	5×10^{30}
	Cyg X1	6	5×10^{30}
	Cyg X2	6	7×10^{28}
Globular clusters	M3	4	3×10^{31}
	M92	6	2×10^{31}
	M15	2	5×10^{31}
Normal galaxy	M31	3	5×10^{35}
Radio galaxy	M87	2	2×10^{37}
Quasar	3C273	3	1×10^{41}

We used a 5 GHz cooled parametric receiver providing a total system temperature of 65 K and an instantaneous bandwidth of 100 MHz. The beamwidth was 10' arc and the sensitivity was ~ 0.1 K Jy⁻¹. The detection instrumentation was based on a single channel high speed receiver, which could be optimised for the range of dispersion measures expected. The backend consisted of an input filter of 10 MHz bandwidth with a characteristic double hump shape to facilitate dispersed pulse identification. A detector with a 2 μ s time constant followed. Pulses were detected by a comparator which compared an adjustable fraction of the instantaneous power with the average power. Pulses were thus detected when the observed temperature exceeded the average system temperature by a preset factor.

These events were recorded in two ways. Initially, a simple pulse duration discriminator was used to discriminate against pulses which were shorter than expected from dispersion, and the remaining events were indicated on a chart recorder. Later in the observing period it became possible to photograph such detected pulses with the aid of a storage oscilloscope and camera, thereby improving our interference and noise rejection capability.

We observed various objects (Table 1) alternating between a 1 h observation on-source and a 1 h observation at an off-source position 1 h later in RA. The event rate was generally near zero, but occasionally rose to ~ 10 h⁻¹ changing on a timescale unrelated to our observing schedule. Inspection of the events recorded on film showed that the faster event rate was caused by pulses with a regular modulation presumably due to terrestrial interference. Almost every other pulse observed could be attributed either to internally generated spikes (characteristic and repeatable shape, associated with the switching of telescope

drive motors) or simply to noise (too short for the expected dispersion and consistent with statistical expectations). For the two sources (Jupiter and Algol) for which no dispersion would be anticipated no events of any timescale were recorded. Only one event looked anything like a real dispersed cosmic signal, and that was on a reference for Cyg X-1.

With the discriminator set at about three times the system temperature, the limiting sensitivity is

$$E_{\text{lim}} = 10^{32} \tau \left(\frac{\nu_c}{5} \right) \left(\frac{D}{100} \right)^2 \text{ erg} \quad (1)$$

with the pulse duration τ in μs , upper cut-off frequency ν_c in GHz, and distance D in kpc. Dispersion in the interstellar medium determines τ , and for our observing frequency and bandwidth $\tau(\mu\text{s}) \approx DM$ (pc cm^{-3}). The dispersion measure (DM) was estimated using a model of the electron distribution in the galaxy^{4,5}, and substituted in equation (1) to give

$$E_{\text{lim}} \sim 10^{32} \left(\frac{\nu_c}{5} \right) \left(\frac{D}{100} \right)^2 \times \left[\frac{19}{\sin b} (1 - \exp(-1.7D \sin b)) + 10^{-4} D \right] \text{ erg} \quad (2)$$

where b is the galactic latitude. The $10^{-4}D$ term allows for additional dispersion by an intergalactic medium with $n_e = 10^{-7} \text{ cm}^{-3}$, but this is only important for the most distant sources. Taking the characteristic frequency to equal the observing frequency (the most optimistic case), the limiting energies for the various sources are given in Table 1.

Observations of all sources listed in Table 1 can be used together with the reference observations to set an overall limit on the rate of 10^{32} erg radio bursts from exploding black holes. Taking the maximum distance sampled in each direction as that for which E_{lim} from equation (2) is 10^{32} erg, we find a limit of $9 \times 10^{-6} \text{ pc}^{-3} \text{ yr}^{-1}$. This compares with the limits derived in the earlier analyses^{3,4} of radio data, and is far superior to limits obtained from searches for γ -ray bursts.

It may be more interesting to consider the globular clusters separately from the other sources. If the primordial black holes have a space distribution similar to that of the visible matter in the Universe, then there would be concentrations of them in globular clusters. Taking $10^5 M_\odot$ as a typical mass for a globular cluster, the limits in Table 1 imply an upper limit of 10^{-20} yr^{-1} for the fraction of the total mass which evaporates due to 10^{15} g black holes exploding and generating 10^{32} erg radio bursts. In other words, primordial black holes of $<10^{15}$ g can never have exceeded 10^{-10} of the total mass.

An even larger mass is sampled in the M31 observations where our beam corresponds to a linear size of 2.6 kpc. However, the additional dispersion in M31 and the greater distance result in a much higher detection limit.

These limits could be improved by using larger telescopes for longer times and simultaneous observations with more than one antenna. Interference rejection in particular would be greatly facilitated by using two or more widely spaced antennas. Large improvements could be achieved either by using a fast multi-channel dedispersing backend or possibly by observing at higher frequencies.

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Photographic amplification of faint astronomical images

METHODS of contrast enhancement in astronomy, such as optical contrasting¹ or optical printing onto a high-contrast material, include all grains throughout the emulsion thickness, and noise (fog), as well as the image, is enhanced. Fairly high levels of chemical fog (up to diffuse densities of 0.3) are common when plates are optimally hypersensitised by baking or prolonged soaking in nitrogen followed by reduction sensitisation in hydrogen gas. Shaw² has shown that chemical fog seriously affects the detective quantum efficiency (DQE) of an emulsion when all the fog grains appear in the image as additional noise. I describe here a method that has been devised to make visible extremely faint images on astronomical plates without imaging fog grains. The images are not apparent on visual inspection and cannot be detected by conventional macro-densitometry. These effects result in a significant improvement in the visual detection of faint images on the original plate over the density range occupied by threshold imagery. The technique is most useful for revealing faint nebulae and low surface brightness features on fine-grain high-contrast emulsions such as Kodak spectroscopic plates type IIIa.

The technique depends on the fact that faint images tend to be located in the uppermost layers of the developed emulsion whereas grains due to chemical fog are distributed at random throughout the total thickness of the emulsion coating. Unlike most film products, spectroscopic plates do not have a transparent protective super-coat to prevent abrasion. The faintest and sharpest images therefore occur at the emulsion-air interface. By using a diffuse-light vacuum-contact printer it is possible to make contact copies of original plates which record the upper layer image only. Fog grains at lower levels are not resolved and add a uniform neutral density which has no effect on the image. The signal-to-noise ratio is thus improved. With sky-limited plates the faint signal is only slightly denser than the uniform exposure from the sky background. The use of a high contrast copy film and careful control of exposure ensure that a very narrow range of densities around the signal level is expanded without the addition of background noise from deeper within the emulsion.

To produce the contact copies a commercially available diffuse-light vacuum-contact printer is used. A simple modification to enable a wide range of original plate densities to be handled has been previously described³. Agfa Gevaert graphic arts film type FO 71p has several characteristics which make it particularly suitable for use with this method. This high-contrast film has an orthochromatic fine-grain emulsion with developer components built into it for use in a stabilisation processing machine. The film can be processed in any alkaline solution but in practice a standard print developer at normal strength gives rapid and uniform results by processing in open trays. Development is effectively complete in less than 2 min at 20°C and consistent results are not significantly dependent on the degree of agitation in the developer solution. Conventional stop bath, fixation and washing complete the processing. Lithographic (infectious development) materials produce too much contrast for this method.

The contact exposure is adjusted so that the true sky background, well away from any nebulae or vignetting, develops to

a diffuse density of 0.4–0.6 on the copy positive. At this point on its characteristic curve the film has a very high gamma, so exposure is critical. The processed copy may be enlarged or contact printed onto a hard grade of paper to give a negative print. Again, exposure is critical and should be arranged so that substantial density is produced in the sky background.

The method is most successful with fine-grain high-contrast plates such as Kodak types IIIaJ and IIIaF. In coarser-grained emulsions faint images are scattered to greater depths within the recording layer⁴ and the contrast in faster emulsions tends to be lower. Copies of plates are less suitable than originals as the relative positions of image and fog grains have been changed, particularly if a collimated or distant point light source was used to make the first copy.

Unlike most optical methods of copying, the technique can be used with plates of any size, limited by the contact printer and available film stock. The largest plates copied to date have been 355×355 mm plates from the UK 1.2-m Schmidt telescope. Dish processing of these large sheets of film is possible because the process is so simple. However, the originals must be of the best quality. Plates taken in very thin haze which appear perfectly acceptable show alarmingly large haloes around the brighter stars when copied as described. The extent of telescope vignetting and internal reflections are likewise clearly revealed as are development defects and marks due to handling and surface abrasions on the original plates.

IIIa plates exposed so that the sky background density reaches about 1.0 (ANSI diffuse) above fog seem to show the faintest

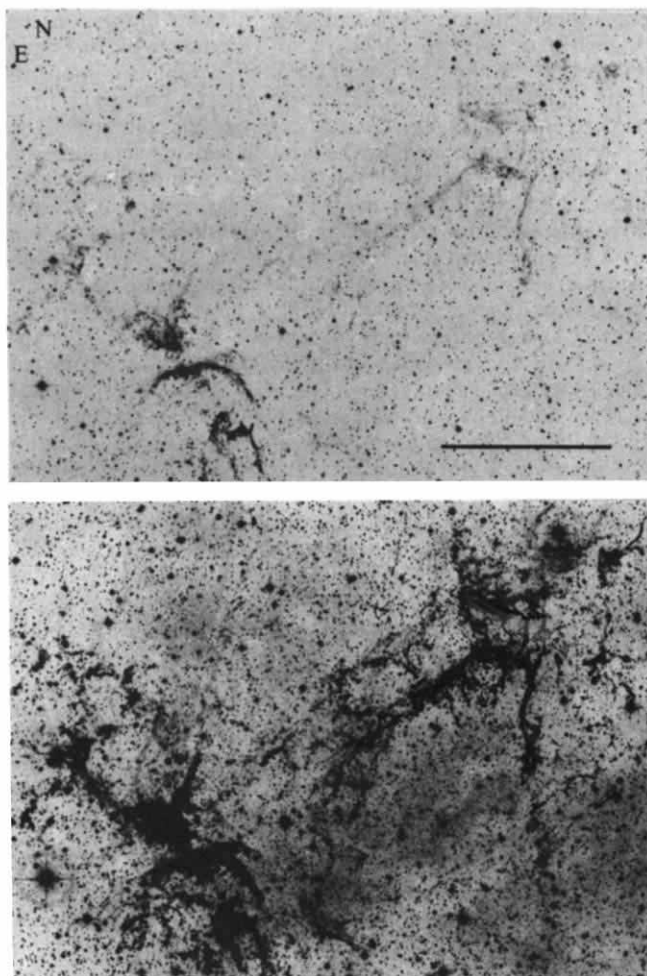


Fig. 1 Faint filaments around Cen A (centre). Inset shows visual appearance of original plate. Both prints have the same scale and orientation and are from the same IIIaJ plate taken on the 1.2-m UK Schmidt telescope. Scale bar, 30 arc min.

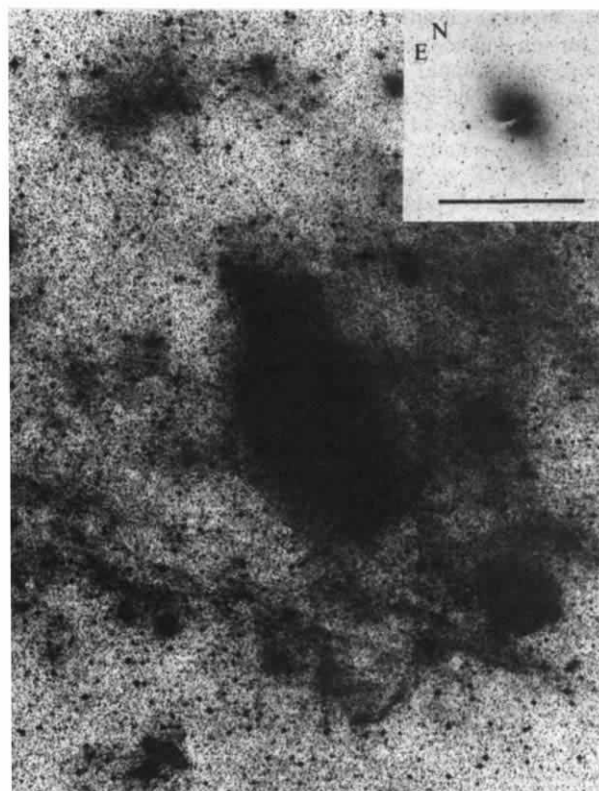


Fig. 2 Part of the Puppis supernova remnant from a deep IIIaF/RG630 plate taken on the 3.9-m Anglo-Australian telescope. A normal copy of the plate (above) is compared with a contrast-enhanced version of the same plate (below) printed with the same scale and orientation. Scale bar, 5 arc min.

objects. In these circumstances all the faint imagery is on the steep part of the characteristic curve where the output signal-to-noise of the emulsion is at a maximum⁵. Figure 1 is a high-contrast derivative of a sky-limited IIIaJ/GG 395 plate of Centaurus A (NGC5128) taken on the UK 1.2-m Schmidt telescope at Siding Spring, Australia. The visual appearance of the original plate is illustrated by the inset, which has the same scale and orientation. Both prints were obtained from the same plate. The NE–SW extension and western loop of Cen A described by Johnson⁶ are almost burned out in this reproduction but much new filamentary material can be seen, particularly to the south and west. The reality of this faint material is confirmed on other high-contrast derivatives made from different original plates; it continues on to adjoining SRC Sky Survey fields. It could well be galactic in origin and not associated with Cen A. Even so, that nebulosity which is obviously associated with Cen A extends along the major axis over a distance of approximately 1.2° .

The quantitative brightness of the faintest objects revealed by the technique is difficult to determine. However, the method seems capable of resolving structure about 0.5% brighter than the night sky (Cannon, personal communication), and Carter (unpublished results) has recently derived a B value, from deep IIIaJ plates of 22.9 mag arc s⁻² for the night sky at Siding Spring. These values imply that the faintest features on the prints have a surface brightness of about 29 mag arc s⁻².

Figure 2 shows part of the Puppis supernova remnant, photographed on a IIIaF/RG630 plate using the 3.9-m Anglo-Australian telescope. Both illustrations have the same scale and orientation and are made from the same plate. The upper print represents the normal visual appearance of the plate but the lower print, made with the amplification technique, shows faint filaments and diffuse background nebulosity not previously observed.

This simple technique has been used with success on objective prism plates, direct and image tube spectra plates and non-limiting exposures on spectroscopic plates from several sources. It can be undertaken with commercially available equipment and materials and requires no special skill in its operation.

I thank Dr R. D. Cannon for permission to publish the deep photograph of Cen A and the staff of the UK Schmidt Telescope Unit for access to original plates.

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Possible identification of the 45- μ m ice signature in Orion

THE composition and size distribution of interstellar dust are controversial matters. Even the ice contribution to the well known and conspicuous 3- μ m feature of the extinction curve has recently been questioned. We now report a study of the middle-infrared spectrum of dust emission. This spectral region is of interest because it includes the 45- μ m band of water ice which, unlike the 3- μ m feature, is characteristic of the ice lattice—being essentially due to stretching vibrations of the H–O bond. The 45- μ m band also carries information on the physical state and temperature of the ice¹. If the ice is amorphous, the band is wide ($\sim 19 \mu$ m) and, at 100 K, peaks at 45 μ m; crystalline ice produces a narrow peak $\sim 2 \mu$ m wide at 43.5 μ m, for a temperature of 100 K; these characteristics are all temperature dependent. Note that the only possible crystalline phase at pressures below 1 bar is Ic (cubic) or Ih (hexagonal), for both of which the above statements are true. The relevant lattice vibrations are essentially translational and probably associated with the transverse optical branch of the dispersion diagram. Our results suggest an emission due to predominantly amorphous ice.

In the simplest astrophysical model, the 45- μ m feature can be observed in emission from hot sources or in absorption through cold dust against a hot background. In the Northern Hemisphere, the bright Kleinmann-Low (KL) nebula is particularly suitable². Observations were made using a grating spectrometer mounted at the Cassegrain focus of a 30-cm telescope on board the C.E.V. Caravelle, with a focal distance of 2,400 mm (ref. 3). Other system characteristics are given in Fig. 1 legend.

The airborne observations were carried out over France on 28 February and 13 March 1978, at a constant altitude of 11,300 m. The outside temperature was -53°C and the dew point -55°C . The pressure was ~ 210 mbar and the line-of-sight elevation remained between 27 and 33 $^\circ$. The telescope was pointed to the KL nebula, offsetting from $\theta^1\text{C}$ in the Trapezium, and the field of view was 4.5 and 1.8 arc min in elevation and azimuth respectively. The latter corresponds to a spectral slit width of 2.5 μ m for an extended source and 1.3 μ m for a point source, if alignment is perfect.

The overall spectral transmission (t_i) of the instrument, including the detector, was calibrated against a black body in the laboratory, before and after the flight. Atmospheric absorption bands were used to calibrate the wavelength scale. From the difference between spectral transmissions obtained at black-body temperatures of 200 and 400 $^\circ\text{C}$, we conclude that the

error on relative transmission values is $<10\%$. The error on absolute values is not relevant to our purposes. Atmospheric transmission (t_{atm}) was computed on the basis of a standard Earth atmosphere⁴ for different moisture contents (w) and spectral slit widths ($\delta\lambda$). A satisfactory fit to the observed spectrum is obtained for 5.2 μ m zenithal precipitable water and $\delta\lambda = 1.5 \mu$ m. The average (I_{raw}) of eight of the recorded spectra, taken in identical conditions (except for the interchange of beams, which eliminates spurious offset signals) was considered as representative of the data, and divided by $t_{\text{atm}} \times t_i$ to give the spectrum (I_{KL}) of the nebula.

In addition to the usual noise sources, our study was affected by fluctuations in the effective wavelength of the detected radiation for a given setting of the grating; this was due to telescope pointing fluctuations. Using the apparently fluctuating locations of the H₂O absorption bands at 35.6, 40.2 and 44.3 μ m, the standard deviation (σ) was $\sim 0.4 \mu$ m (corresponding to a pointing deviation of 0.7 arc min). As a consequence, averaging over several spectra leads to an apparent widening of the slit, which was taken into account in computing t_{atm} .

Figure 1 represents the spectrum of the KL nebula, I_{KL} , together with that, I_b , of a body at 80 K having an emissivity proportional to λ^{-1} . The latter is very close to the spectrum of a grey body at 100 K and both fit reasonably well experimental data obtained at lower resolution over a wider band^{2,5}. Our data are similar to those obtained by Ward⁵ in the same range. Both studies indicate a bump at about 43 μ m. If this is attributed to the amorphous ice feature, with a peak at $\lambda_0 = 43 \mu$ m and a FWHM $\delta\lambda = 19 \mu$ m, then, trying to fit a function of the form

$$I_b \left\{ A + B \exp \left[-4 \ln 2 \left(\frac{\lambda - \lambda_0}{\delta\lambda} \right)^2 \right] \right\}$$

through the data points gives $A \approx 1$, $B \approx 0.4$, corresponding to the dashed curve in Fig. 1. This set of values is obtained assuming a constant r.m.s. noise voltage over the spectral range and the validity of the fit is confirmed by the χ^2 -test (reduced $\chi^2 \approx 1$). The error bar at 43 μ m in Fig. 1 represents the relative standard deviation of the fit ($\pm \sigma/A$). (At 43 μ m, this is equivalent to a r.m.s. noise voltage of 120 nV Hz^{-1/2} on the detector, per spectral element). The choice of the exponential form for fitting was made for lack of a better knowledge of the ice 45 μ m band profile in terms of dust extinction. It was intended to assess the objective existence of a feature near

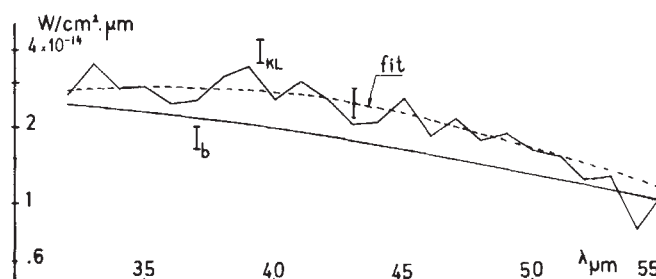


Fig. 1 I_{KL} , average measured spectrum of KL nebula, corrected for instrumental and atmospheric transmission. I_b , grey body

spectrum, $T = 80 \text{ K}$, $\epsilon = \frac{30}{\lambda (\mu\text{m})} \times 0.1$. Dashed curve, least squares fit to I_{KL} . Error bar $\pm \sigma/A$, where σ is the standard deviation of the fit (see text). Spectrometer system characteristics: aluminium echelette grating, ruled at 20 lines per mm, $20 \times 20 \times 6 \text{ mm}$ in size blazed at 42 μ m, in a folded Czerny–Turner design. The entrance hole is circular, 3.2 mm diameter. Average system transmission: 3%; detector is a Low bolometer, with BaF₂ Reststrahlen filters. The grating is driven by a synchronous motor, through a gearbox at an average speed of 0.1 $\mu\text{m s}^{-1}$ between 30 and 60 μ m. The telescope secondary is wobbled at 20 Hz, with a sinusoidal beam throw of 3 arc min.

45 μm , which it does indeed, since B/A (40%) is clearly larger than σ/A (17%), notwithstanding possible errors in instrumental spectral response (<10%).

The data therefore seem to indicate the presence of an emission due to predominantly amorphous ice. Its relative amplitude (B/A) is, however, much smaller than predicted for an optically thin cloud permeated with 'standard' core-mantle grains^{6,7}. Since KL is indeed thin in the wavelength range considered ($\tau \sim 0.1$, from the comparison of colour and brightness temperature), the data would point to a shell model where the ice mantles are only present at the periphery of the nebula⁶. A satisfactory fit of such a model to the experimental data still requires proper tailoring of grain dimensions and densities, shell radii and central heating source.

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Atomic oxygen emission at 63 μm as a cooling mechanism in the thermosphere and ionosphere

FAR IR emission by atomic oxygen fine structure transitions is considered an important cooling mechanism in the Earth's upper atmosphere and ionosphere as well as in several astronomical objects. The cooling rates so far have only been calculated theoretically on the basis of local thermodynamic equilibrium. No experimental or theoretical determination of the fine structure excitation or quenching cross sections for mutual neutral atomic oxygen collisions has been reported. We report here the first spectrometrically measured atomic oxygen 63- μm emission profile at thermospheric altitudes (90–180 km). An energy-averaged quenching cross section for the $\text{O}(^3\text{P}_1)$ level was deduced. The importance of the 63- μm emission as a cooling process for both the neutral thermosphere and the ionosphere is much less than predicted.

On 16 March 1977, at 2304 CET a liquid helium cooled far IR grating spectrometer was launched aboard a Skylark 7 rocket from Andoya, Norway, as part of the payload AI/II-PHA-F4D of the 'Polar High Atmosphere' rocket campaign (see ref. 1 for

details). During the flight the geomagnetic field was moderately disturbed (200 nT). Measurements were taken in the wavelength interval of 45–125 μm .

Figure 1 shows preliminary absolute zenith intensities of the 63- μm line measured by this experiment (code name AB3). The data are compared with a model calculation by Kockarts and Peetermans² (KP1 and KP2). This model includes radiative transfer and is valid under the following assumptions: (1) the atomic oxygen is in diffusive equilibrium; (2) the fine structure level occupation densities obey a Boltzmann distribution at the local gas kinetic temperature, that is, local thermodynamic equilibrium (LTE) is assumed; (3) no additional non-thermal excitation processes are accounted for. Two curves from this model are shown in Fig. 1: curve KP1 assumes an atomic oxygen number density of $1 \times 10^{11} \text{ cm}^{-3}$ at 120 km; curve KP2 is valid for a corresponding value of $5 \times 10^{10} \text{ cm}^{-3}$. The exospheric temperature is 750 K for both curves, close to the exospheric temperature of 800 K at the time of the flight. At low altitudes the 63- μm line becomes optically thick. The agreement between the measurement and the model curve is quite good at these altitudes, taking into account experimental error (bar) and approximations used for the model calculation. At higher altitudes, however, the measured intensities are much lower and exhibit a much steeper altitude gradient than the calculated ones. Neutral gas densities, including atomic oxygen, were measured by a mass spectrometer during the upleg of the rocket trajectory (E. Hettmannsperger, P. Lämmerzahl and D. Krankowsky, personal communication). The slope of the measured atomic oxygen density profile does not show any substantial departure from a diffusive equilibrium density profile for a 800-K exospheric temperature. Despite local and temporal differences between the upleg and downleg trajectory, the model assumption of diffusive equilibrium seems satisfactory in the conditions of this experiment.

The steep gradient of the measured 63- μm line intensities can be understood if the atomic oxygen line is not in LTE. The atomic oxygen concentration in the $^3\text{P}_1$ state may then be described by

$$n_j = n(\text{O}) \frac{f_j g_j \exp(-E_j/kT)}{\sum f_j g_j \exp(-E_j/kT)} \quad J = 2, 1, 0 \quad (1)$$

where $n(\text{O})$ is the total atomic oxygen number density, g_j the statistical weights $2J+1$ of level J , E_j the energy above the P_2 ground level, k Boltzmann's constant, T the local gas kinetic temperature, and f_j the deviation from a Boltzmann distribution. The functions f_j are determined as a function of altitude by solving the steady-state equation for the three fine structure level densities. The steady-state equation includes spontaneous and induced emission, reabsorption of radiation and collisional activation and quenching. A first approximation of the solution is obtained by neglecting any effects caused by collisional and radiative transitions to and from the $^3\text{P}_0$ level.

Two curves, NB1 and NB2 in Fig. 1, were calculated for a non-LTE distribution of the fine structure level densities. Curve NB1 was derived from the density and temperature conditions of the KP1 profile. Curve NB2 is valid for the corresponding conditions of the KP2 profile. The specific radiation intensity needed for solving the steady-state equation was derived from the measured 63- μm intensity in the optically thick altitude regime and was assumed to be altitude independent. Both curves fit the measured altitude gradient reasonably well. An energy-averaged quenching cross section of about 10^{-20} cm^2 is adopted to produce these curves. Best agreement with the measured intensities is obtained with the profile NB1. Increasing the quenching cross section by a factor of 2 already causes a considerable discrepancy between measured and calculated profile gradients. Still lower cross sections are possible, however, as the curve approaches a radiation equilibrium profile. The value of 10^{-20} cm^2 is therefore an upper limit at this early state of the data analysis.

Atomic oxygen fine structure levels may also be excited by electron impact. This process is thought to act as an important

cooling mechanism for ionospheric electrons³. In this case electron densities and temperatures were measured simultaneously on the same payload (A. Dumbs and E. Neske, personal communication); atomic oxygen excitation by electrons was therefore included in a second step in the steady-state equation. The electron energy loss rate given by Dalgarno⁴ was used and divided by a factor of 2 following a suggestion by Hoegy⁵. The 63- μm line intensities calculated for thermal and electron impact excitation in non-LTE conditions based on the profile NB1 are shown in Fig. 1 (NE1). The difference between the values of the curve NE1 and NB1 shows the effect of the additional electron excitation. It seems that the cross sections for electron impact excitation of atomic oxygen fine structure levels by Hoegy⁵ are still too high by a considerable factor. The detailed evaluation of the interplay between thermal and electron excitation of the 63- μm line is now underway.

lower electron to atomic oxygen excitation cross sections as described here would help to lessen this discrepancy.

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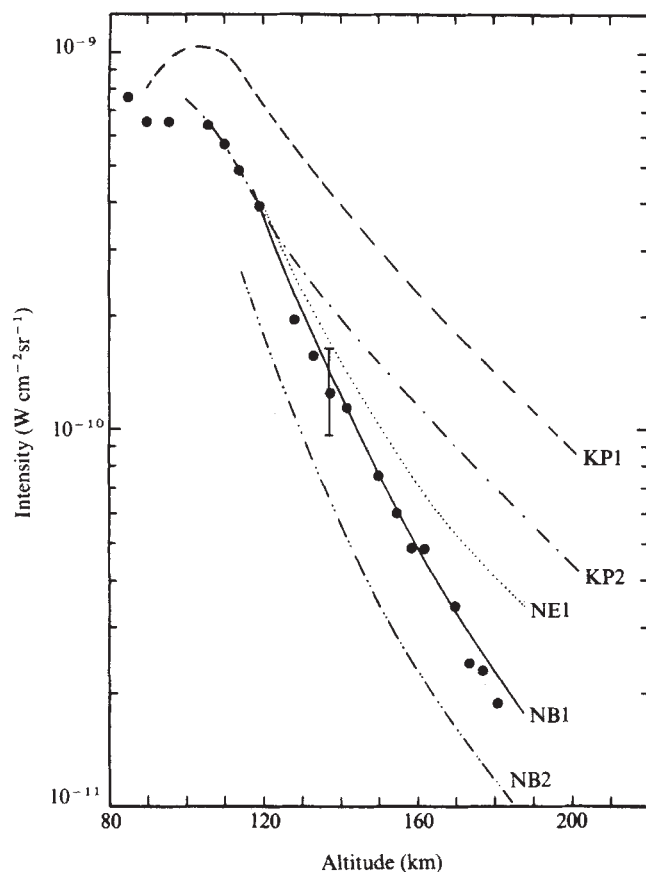


Fig. 1 Atomic oxygen 63- μm zenith intensities. ●, Individual data obtained during downleg of rocket trajectory; other labels, see text.

The preliminary 63- μm emission profile reported here has considerably lower emission rates than predicted and the importance of the 63- μm emission as a cooling process for the neutral thermosphere is much less than previously assumed. Measurements have enabled the determination of at least an upper limit of the fine structure quenching cross section for O-O collisions and the deduced value of 10^{-20} cm^2 is several orders of magnitude less than was previously assumed. The altitude dependence of the electron cooling rates as found experimentally⁶ does not agree at all altitudes with the theoretical expectations for the energy transfer from electrons to neutral atomic oxygen directly or by ions. Lower 63- μm emission rates and

Helium gas bubble lattices in face-centred-cubic metals

ION irradiation of metals with helium or hydrogen isotopes commonly results in the formation of microscopic gas bubbles. At very high doses, radiation blistering and related phenomena can cause severe damage to surfaces¹. Much research has been stimulated by the need to find materials with acceptable erosion and gas loading properties for use in future controlled thermonuclear reactors. Bubble formation is also important in fission-reactor fuel technology and other applications^{2,3}. The underlying bubble structure of face-centred-cubic (f.c.c.) metals has remained largely unknown, although much has been done on depth profiling light gases implanted to high doses (see, for example, refs 3-5). Inert-gas bubbles lying on a space lattice have been reported previously only for the body-centred-cubic (b.c.c.) metal molybdenum^{6,7}. There have been many observations, mainly for b.c.c. metals, of the similar ordering of voids produced by particle irradiation^{8,9}. The void lattice spacing is typically 5-10 times that found for bubbles ($\sim 5 \text{ nm}$). We report here on the bubbles produced in several f.c.c. metals by 30 keV helium-ion irradiation to a level just below the critical dose for blistering. A new result for this important class of metals is that the helium gas bubbles lie on a superlattice having an f.c.c. structure with principal axes aligned with those of the matrix.

Annealed polycrystalline targets electropolished to a high surface finish were irradiated at room temperature to a level of approximately $4 \times 10^{17} \text{ He ions cm}^{-2}$ with the ion beam normal to the surface. The main study was of copper, for which the mean projected range and r.m.s. spread in projected range, calculated using the Harwell version¹⁰ of the computer code E-DEP-1 of Manning and Mueller, are 97 nm and 43 nm respectively. To ensure that the samples were all at approximately the same stage of bubble development, an ion beam having a lateral gradation in current density was used. Radiation blisters formed first where the current density was highest³ and the region of the target which was close to blistering could be easily identified. Samples were taken from this area and thin sections containing the bubble layer prepared for transmission electron microscopy.

Bubbles in copper, typical of those observed for helium-ion irradiation at 300 K, are shown in Fig. 1. Considerable bubble alignment can be seen with dense-packed rows parallel to matrix {111} trace directions. Diffraction from the bubble array caused the splitting evident around the matrix reflections in the electron diffraction pattern of Fig. 2. The strongest reflections in the superlattice pattern are assigned (111) indices as they are in line with the (111) matrix spots and are in the positions expected for diffraction from the dense-packed planes seen edge-on as bubble rows in Fig. 1. The results show that the bubble-lattice is at least predominantly an f.c.c. structure aligned with the copper matrix. The bubble diffraction patterns for the matrix orientations [100], [111] and [112] support this conclusion. Alignment conforms, as expected, to crystallographic features in the

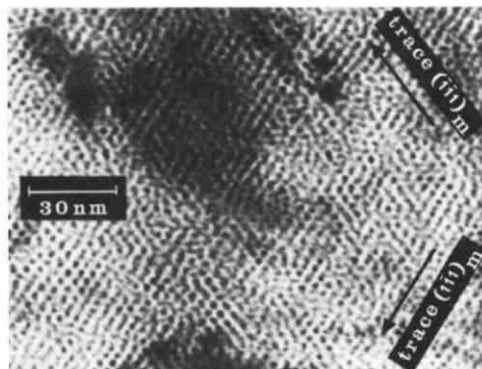


Fig. 1 Direct transmission electron micrograph of helium gas bubbles in copper following helium-ion irradiation to a level of $\sim 4 \times 10^{17}$ 30 keV He ions cm^{-2} at 300 K. The electron beam is along [110] in the matrix and is normal to the plane of the bubble layer. The directions of the traces of the (111) matrix planes in the (110) plane are indicated. The bubble lattice constant, a_b , found from 380 measurements of inter-bubble spacing is 7.7 ± 0.8 nm.

copper; bubble rows run parallel to matrix slip traces and assume the twin-direction on crossing twin boundaries.

The lattice constant found, $a_t = 7.6 \pm 0.3$ nm, corresponds to approximately 10^{19} bubbles cm^{-3} . There is good agreement between the results both for samples from the same irradiation and for the several different copper targets used. The lattice constant based on diffraction from a given set of planes is consistent for the above orientations. There are, however, small but apparently significant differences between the different planes. For example, the {200} planes give a consistent value for a_t of 6.8 ± 0.4 nm. Some variations in structure are apparent and will be reported elsewhere. Direct micrographs at [110] show domains of good ordering typically of five inter-bubble spacings across with regions between of comparable size in which the ordering is less obvious.

The average bubble diameter found using a through-focus technique is 2.0 ± 0.5 nm. The ratio of lattice spacing to bubble radius is in the range of values found for void lattices⁹. This

suggests there may be a common mechanism of lattice formation. If all the implanted helium is contained in bubbles of the size and separation measured here, then it can be estimated that the bubbles may be over-pressured by a factor of 5 or more. The difficulty is in relating helium pressure to atomic density at very high densities¹¹. Over-pressure is a necessary element in a recent theory of blister formation¹¹. The associated volume swelling is 5%; the swelling could be higher and the degree of over-pressure less if there were an additional large population of small gas bubbles below the resolution limit of the microscope (~ 1.5 nm for cavities in copper). We have been unable to detect this either in diffraction at 300 K or in direct microscopy on specimens heated to induce bubble growth. For this reason the existence of a significant population is thought to be unlikely. The bubble lattice seems to form only at high dose ($\geq 2 \times 10^{17}$ He ions cm^{-2}), but once formed it is stable at temperatures up to approximately $0.3 T_m$. Following blistering at 300 K, a bubble lattice with similar parameters has been seen here to survive in some blister caps as well as in the region between blisters.

Similar helium-bubble lattices are seen after irradiation at 300 K in the two other f.c.c. metals studied, nickel and AISI 321 (18Cr: 10Ni: Fe) austenitic stainless steel. The lattice constants based on diffraction from (111) superlattice planes are 6.6 ± 0.5 nm and 6.4 ± 0.5 nm respectively. At 300 K copper is above the vacancy mobility temperature while nickel and stainless steel are below. As in molybdenum, vacancy mobility does not seem to be a major factor in forming the lattice, at least in the limited range of low mobility covered by these metals. In this context it is interesting that considerable bubble alignment has been observed in the present experiments in proton-irradiated copper where the rate of vacancy production by the primary beam is a factor of 10 less than for helium-ion irradiation.

The reproducibility of the results suggests that bubble lattice formation could be a common feature of high-dose inert-gas implantation in annealed f.c.c. metals at low temperature. P.B.J. thanks The Nuffield Foundation for financial assistance.

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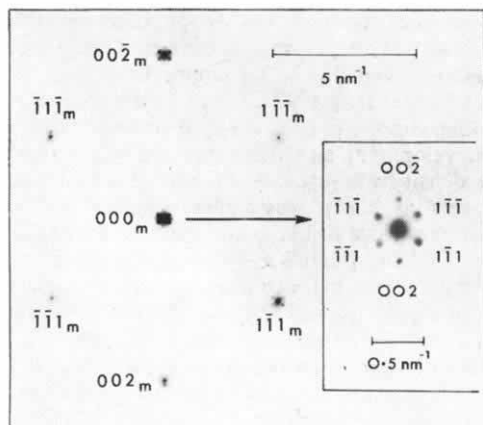


Fig. 2 A selected-area electron diffraction pattern from the specimen of Fig. 1. The electron energy is 100 keV. The matrix pattern is shown on the left and the superlattice diffraction pattern centred on the (000) transmitted beam is shown enlarged in the inset. In the latter slight changes in specimen tilt and translation have been made to obtain more uniform excitation. The value of a_t deduced from the separation of the (111) spots is 7.6 ± 0.3 nm.

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Crown ether intercalations with phyllosilicates

It has been discovered in the past decade that some macrocyclic polyethers (crown ethers) act as ligands to form stable complexes with salts of numerous metals. These are the first synthetic compounds able to coordinate to alkaline cations¹⁻³.

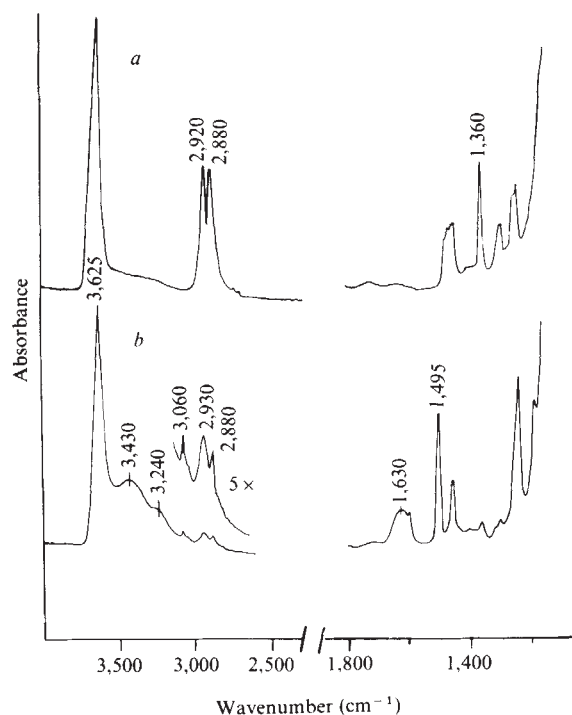
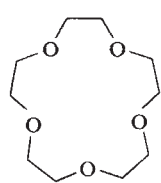
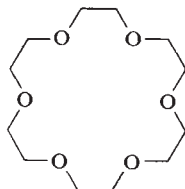


Fig. 1 Infrared spectra of Ba-montmorillonite treated with a methanolic solution of: a, 15-crown-5; b, dibenzo 18-crown-6.

The cyclic structure of the crown ethers creates a circular cavity of oxygen atoms to which the cations coordinate. The diameter of this cavity as well as the relative size of the cation, determine the stability of the resulting complexes, and the cationic selectivity depends on it. The most studied crown ethers are derived from so called 15-crown-5 and 18-crown-6:



15-crown-5



18-crown-6

We have shown experimentally and report here that the crown ethers are able to intercalate between the layers of the 2:1 phyllosilicates, saturated with alkaline or alkaline-earth cations, showing an unusual stability. The complexes were obtained mainly by immersion of homoionic montmorillonites (Wyoming bentonite), in different solutions of crown ethers. Hectorite and vermiculite were also tested with positive results.

Many neutral organic substances, such as alcohols, ketones, phenols, and aromatic hydrocarbons, can intercalate the interlamellar space of clays of the smectites group⁴⁻⁵. Generally, the fixation of these compounds is due to their interaction with the exchangeable cations, which balance the deficit of charge of the silicate layer. Nevertheless, the stability of those complexes is relatively low, especially in the presence of alkaline cations. Water molecules also easily replace the organic ligands, by exposition to atmospheric moisture.

The coordination between the interlamellar cation and the crown ether, can be shown by the UV spectra of montmorillonite treated by dibenzo-18-crown-6. The band at

275 nm, due to the aromatic ring, is displaced in the Na-, K-, and Ba-montmorillonite complex, to 285 nm. A shift of 6 nm has been observed in complexes of the same crown ethers with different inorganic salt solutions. The shift was explained by the bathochromic effect of the cation associated to the aromatic ring¹.

Table 1 Values of the increase in the basal spacings (Δd) in homoionic montmorillonites (M-K⁺, M-Na⁺, M-Ba⁺⁺) treated by some crown ethers.

Crown ethers	M-Na ⁺	Δd_{001} (Å) M-K ⁺	M-Ba ⁺⁺
12-crown-4	8.1	4.4	6.6
15-crown-5	4.0	4.4	8.0
18-crown-6	6.3	4.4	3.9
dibenzo-18-crown-6	8.9	7.9	7.7
dibenzo-24-crown-8	8.2	9.0	7.1

The basal spacing (d_{001}) of the anhydrous montmorillonite is 9.6 Å.

The IR spectra show that the coordination of the crown ether with the interlamellar cation produces a displacement of water molecules which originally belonged to the sphere of coordination of the cation. Figure 1a shows the IR spectrum of the Ba-montmorillonite complex 15-crown-5 where the H₂O bands have disappeared. However, when crown ethers of a larger size are intercalated, the steric effect prevents the complete association to the cations, and the water molecules are not completely displaced. Figure 1b shows the spectrum of Ba-montmorillonite/dibenzo 18-crown-6 complexes, in which the bands at 3,430 and 3,240 cm⁻¹, ν (OH) stretching, and the 1,630 cm⁻¹, δ (HOH) bending are visible but less intense. The displacement of water molecules is irreversible, in contrast with the already mentioned complexes with other neutral organic molecules.

The intercalation of the crown ethers in phyllosilicates can be also followed by X-ray diffraction. In most cases the increase of the basal spacing is either 4 or 8 Å (Table 1), which may be interpreted as monolayer or two-layer complexes respectively. The macrocyclic ligands are arranged parallel with respect to the silicate layers, in agreement with the thickness of the crown molecules. The 001 reflections present integral orders, suggesting a reasonable crystalline order for most of the montmorillonite complexes such as 15-crown-5, 18-crown-6 and its derivatives. In some cases interstratifications are obtained.

The high stability of these new complexes is the most interesting feature: the crown ether is not desorbed by water treatment, organic solvents or salt solutions. In salt solutions the complexes can maintain the cation exchange capacity of the original mineral. Besides, thermal treatment is not able to desorb the crown ethers, as shown by the exothermic peak around 350 °C, observed by DTA.

Note added in proof: Intercalation complexes of phyllosilicates saturated with alkaline, alkaline-earth or transition metal cations have been recently prepared in our laboratory with polyoxadiazamacrobicyclic ligands ('cryptands').

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Measurement of thermal maturation of petroleum by proton magnetic resonance spectroscopy

THE processes of petroleum generation and maturation have been shown to depend on the thermal breakdown of organic material contained in the sediments as the temperature increases due to increased burial depth^{1,2}. Measurement of the degree of thermal alteration of the organic matter in shales and other potential petroleum source rocks is therefore of fundamental importance to petroleum exploration. The most common methods of estimating the degree of thermal alteration are based on measurements of the residual insoluble organic material in rocks. Some of the more common methods use changes in the chemical composition of the kerogen³, electron spin resonance³, kerogen coloration⁴ and vitrinite reflectance⁵. The method reported here is based on measurements on the soluble aromatic hydrocarbons extracted from the sediments. As the aromatics are represented in both the source rock and reservoir petroleum they may eventually provide a useful medium for comparing the thermal history of reservoir and source rock hydrocarbons. This method uses well established chemical techniques and provides a simple quantitative measure of thermal maturation by measuring the percentage of aromatic protons in the aromatic fraction of petroleum.

Samples of petroleum-bearing shales and siltstones were obtained from drilling operations in the Carnarvon Basin of Western Australia. Samples from different locations and depths of a uniform sequence of Cretaceous marine sediments were studied. Geochemical data for the samples are shown in Table 1. Each sample was crushed and the soluble organic material was extracted with methylene chloride: methanol (20:1) under the influence of ultrasonic vibration. After careful distillation of the solvent at atmospheric pressure the residue was chromatographed on silicic acid. Consecutive fractions were eluted from the column with pentane, ether and methanol. The fraction eluted with ether was retained as aromatic hydrocarbons and analysed by proton magnetic resonance spectroscopy.

The signal from the aromatic protons (δ 6.7–7.9) was integrated and expressed as a percentage of the total proton integral. This value, the percentage of aromatic protons in the aromatic fraction, will be referred to as the PAP value of an oil or extract.

Although thermal evolution of organic matter depends on both time and temperature, the time factor has been taken as constant for all samples and the PAP values have been plotted against the present sediment temperatures (Fig. 1). The

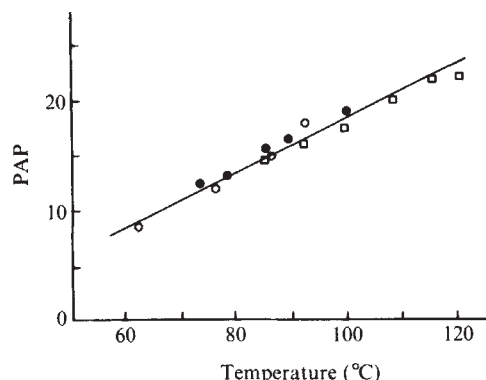


Fig. 1 PAP values plotted against the sediment temperature. Sets of samples from different wells are indicated. ●, Well BH; ○, well BS; □, well BW.

assumption that each set of samples has a similar thermal history is supported by geological evidence that subsidence occurred at each location in the basin over approximately the same time interval, and that the sediments are now at their maximum depth of burial⁶. Samples from three different wells over a temperature range of 60 °C show a regular increase in their PAP value as the temperature increases. This correlation of temperature and PAP value is attributed to the effect of thermal processes on the petroleum and is supported by pyrolysis studies of low maturity hydrocarbons. Figure 2 shows PAP values plotted against time for petroleum which had been subjected to pyrolysis at 300 °C for various times. In these experiments, the low maturity petroleum was contained in a matrix of shale and pyrolysed in evacuated glass tubes. Blank experiments, without added petroleum, showed that the contribution from pyrolysis of hydrocarbons already present in the shale was negligible.

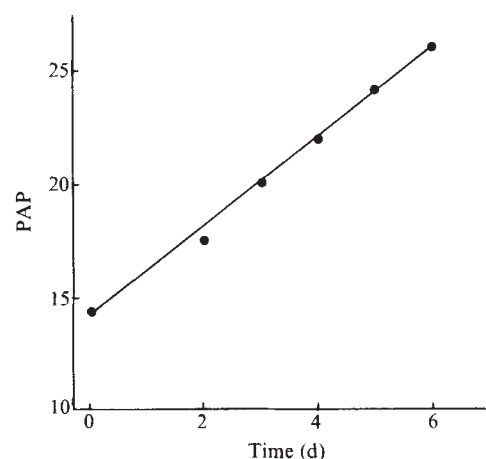
These experiments showed that the changes in the PAP value which result from pyrolysis of an immature petroleum are similar to those which are observed in nature as the sediment temperature increases. It is therefore reasonable to conclude that the variation in PAP value of sediment extracts reflects the level of maturity of organic material in the sediment.

Vitrinite reflectance data for one set of samples are shown in Table 1. Both the PAP and vitrinite reflectance values are consistent and indicate that the organic material has undergone

Table 1 Geochemical data

Sample	Depth (m)	Sediment temperature (°C)	PAP	Vitrinite reflectance (%)	CPI (C ₂₃ –C ₂₉)
BH 1.1	1900	73	13.3	0.63	1.22
BH 1.2	2100	78	13.7	0.72	1.48
BH 1.3	2300	85	15.6	0.82	1.17
BH 1.4	2400	89	16.5	0.86	1.20
BH 1.5	2600	99	18.8	0.95	1.23
BS 1.1	1600	62	8.5	—	0.86
BS 1.2	2000	76	12.0	—	1.34
BS 1.3	2300	86	14.9	—	1.24
BS 1.4	2500	92	17.9	—	1.18
BW 2.1	2260	84	14.4	—	0.96
BW 2.2	2440	92	16.0	—	1.01
BW 2.3	2640	99	17.4	—	0.94
BW 2.4	2840	108	19.8	—	0.99
BW 2.5	3140	120	21.9	—	1.02

Fig. 2 PAP values plotted against time for pyrolysis of an immature petroleum on shale at 300 °C.



moderate levels of thermal maturation. The samples investigated differed in their CPI values and in the proportions of *n*-alkanes and naphthenes. This suggests that this new technique is useful for samples containing a range of chemical types; however, further work is required to fully assess the validity of the technique for a greater range of kerogen types.

This technique may be a useful supplement to existing methods of measuring thermal maturation as it provides a sensitive measure over the small but important temperature range where liquid petroleum is generated and matures.

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Oxidation state of plutonium in the Irish Sea

SEVERAL of the chemical parameters needed to understand the distribution and behaviour of plutonium in the aquatic environment have not been fully investigated in the field. We consider here two of the more fundamental of these parameters: (1) the oxidation state(s) of the plutonium present in seawater; and (2) the extent to which each oxidation state is associated with suspended particulate matter. A widely accepted analytical technique is adapted to separate the two oxidation states expected to show a high affinity for particulate matter, Pu(III) and Pu(IV), from the more soluble pair, Pu(V) and Pu(VI), and is applied to a series of seawater samples. It was found that in the area studied, generally >75% of the plutonium in solution, and <10% of the plutonium on particulate matter, was Pu(V+VI); consequently the distribution coefficients for Pu(III+IV) exceeded those for Pu(V+VI) by more than two orders of magnitude.

Based on comparisons with natural chemical analogues, the different oxidation states of plutonium are expected to show markedly different biogeochemical properties. Uranium and thorium, for example, are very similar in basic chemical properties to Pu(VI) and Pu(IV) respectively. Although the crustal abundances of the two elements are similar, uranium is present in open ocean seawater at a concentration 10^4 times higher than thorium¹. Similarly, differences in direct biological accumulation are observed and, as Cherry and Shannon state², "thorium must, clearly, be taken up more efficiently by marine organisms than is uranium".

The potential importance of oxidation state in determining the environmental chemistry of plutonium has frequently been stated³⁻⁶. Analytical procedures to separate the oxidation states have been published⁷⁻⁸ and applied to laboratory samples. Laboratory experiments have also used plutonium, in initially specified oxidation states, to test the stability⁹, biological availability¹⁰⁻¹³ and attachment to sediments¹⁴ of various oxidation states. These observed biogeochemical differences between

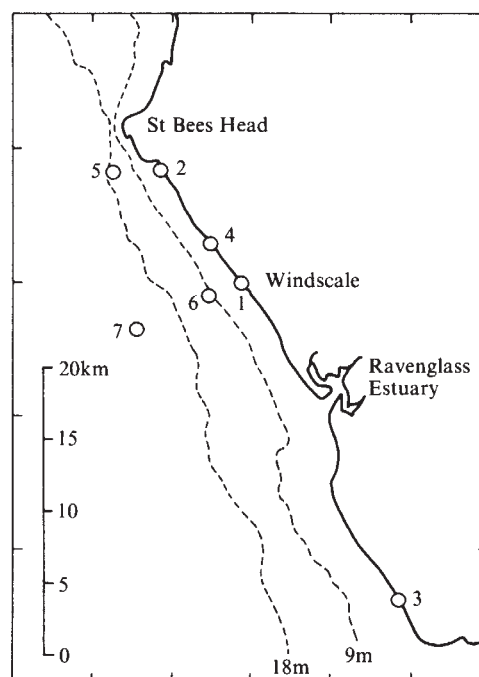
the chemical analogues of plutonium combined with the differences observed between actual plutonium oxidation states in laboratory experiments emphasise the importance of establishing the oxidation state(s) of plutonium found in the environment. However, we know of no information published regarding the actual oxidation states of plutonium found in natural seawater.

The BNF reprocessing plant at Windscale, Cumbria, discharges plutonium into the Irish Sea through a submerged pipeline terminating 2.4 km offshore. This release supports plutonium concentrations of about 1 pCi l^{-1} in filtered seawater near the discharge¹⁵ and provides an excellent experimental area in which to study the oxidation state(s) of plutonium.

Eight seawater samples were collected from the seven locations marked on Fig. 1 during the summer of 1977. Shoreline samples were collected from water depths of ~0.5 m; offshore samples were collected either within 1 m of the surface or within 1 m of the seabed.

For each sample, the association of different oxidation states of plutonium with suspended particles was determined by measuring the concentration of each oxidation state in subsamples processed: (1) without filtration; (2) after filtration through a 0.22 μm Millipore membrane filter; and (3) in five of the eight samples after filtration through both 0.22 and 0.025 μm Millipore membrane filters. The method used for oxidation state differentiation was an adaption of the classical separation of Pu(III) and Pu(IV) from Pu(VI) by the co-precipitation of the former pair on a rare earth fluoride¹⁶. An internal measurement of the completeness of separation was provided by adding tracers of the relevant oxidation states to each sample and then monitoring the degree of separation of these tracers. Each subsample was made 0.8 M in HNO_3 , 0.25 M in H_2SO_4 , 0.5 mM in $\text{K}_2\text{Cr}_2\text{O}_7$ and 0.7 mM in $\text{Nd}(\text{NO}_3)_3$. Unfiltered subsamples stood overnight at this stage to allow exchangeable plutonium to leach from the particles before they too were filtered. Initial observations had shown that the leachate constituted >90% of the total plutonium originally on the particulate matter. Sufficient HF was added to raise the total fluoride concentration to 0.25 M and the resultant NdF_3 precipitate was removed by filtration. $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ was added to the filtrate

Fig. 1 Location of water sampling stations.



(5g l⁻¹) which then stood overnight to complete the reduction of any Pu(VI) in the sample. NdF₃ was again precipitated by the addition of 100 mg of Nd per litre and removed by filtration. The method was considered sufficient to disrupt any complex ions—organic or inorganic—initially present in the sample. The plutonium in each NdF₃ precipitate was purified by routine¹⁷ techniques of anion exchange and individual plutonium isotopes were determined by α -spectrometry using silicon surface barrier detectors.

Completeness of the oxidation state separation and chemical yields were determined from the recovery of two isotopic diluents, ²³⁶Pu(VI) and ²⁴²Pu(IV), added to each subsample as soon as possible after collection (and after filtration for filtered subsamples). Each tracer was isotopically pure and contained >99% of its plutonium in the specified oxidation state. Overall chemical yields exceeded 80% and the separation of the two tracers typically exceeded 97%, even for the unfiltered subsamples. This maximum 3% deviation includes any plutonium which changes oxidation state during the analysis as well as any which simply carries on the incorrect precipitate.

Any Pu(III) or Pu(V) initially present would be rapidly oxidised by the Cr₂O₇²⁻ to Pu(IV) and Pu(VI) respectively and would be indistinguishable from the original Pu(IV) and Pu(VI). The oxidation of Pu(IV) by dilute Cr₂O₇²⁻ is an extremely slow process and was unimportant during these experiments. In a well oxygenated, high pH system, such as surface seawater, the higher oxidation state in both the Pu(III):Pu(IV) and Pu(V):Pu(VI) couples were assumed to be the more likely and subsequent results are reported as Pu(IV) and Pu(VI). A more detailed description and discussion of the method will be presented elsewhere.

The Pu(IV) and Pu(VI) concentrations, tabulated by particle size class in Table 1, were calculated for each sample using the plutonium concentrations measured in the subsamples. The concentration of plutonium on particles >0.22 μ m was the numerical difference between the concentrations measured in the unfiltered subsample and the 0.22 μ m filtered subsample. The concentration on particles between 0.025 and 0.22 μ m was the difference between the concentrations measured in the two filtered subsamples. The concentration in water passing the 0.025 μ m filter (0.22 μ m in the initial three samples) is presented directly. These results indicate that plutonium retained on the filters is predominantly Pu(IV) and that plutonium passed

in the filtrate is mainly Pu(VI). Simply filtering these samples provided a fair separation of the oxidation states, a feature which could prove useful in estimating the oxidation state present in previous measurements. Little of the plutonium of either oxidation state which passes a 0.22 μ m filter is removed by the 0.025 μ m filter, suggesting that the plutonium may be in true solution and not simply attached to very small particles. Furthermore, the ratio of Pu(VI) to Pu(IV) in solution, particularly for the offshore surface samples, is sufficiently constant to suggest first that the two are at or near oxidation–reduction equilibrium, or second, that the rate constants governing the approach to this equilibrium are slow compared with the initial rate of dispersion of Windscale effluent in the sea.

Assuming that there are heterogeneous exchange reactions for both Pu(IV) and Pu(VI) between seawater and suspended particulates, distribution coefficients (K_d = pCi kg⁻¹ in particles/pCi l⁻¹ in water) for each oxidation state as well as for total plutonium were calculated. These K_d values for each sample are given in Table 2. As the composition of suspended particulates is extremely variable, it is not surprising that the distribution coefficients are far from constant. However, the general trends are unmistakable. Although the distribution coefficients measured for total plutonium are typical of values previously reported for the Irish Sea¹⁵ and other systems¹⁸, in these samples the distribution coefficients for Pu(IV) exceed those of Pu(VI) by factors of up to 1,000. This is consistent with the behaviour of the chemical analogues, Th(IV) and U(VI).

Because of this large difference in the K_d value, the initial distribution of plutonium between soluble and particulate phases (including the seabed) in the sea should be highly dependent on the ratio of oxidation states in the source material. It has been observed at Windscale that approximately 95% of the plutonium discharged is rapidly removed to the seabed and that the remaining 5% is removed from the area by water transport in a manner indistinguishable from the transport of the soluble isotope ¹³⁷Cs (ref. 15). From the above data we suggest that this 5% is largely Pu(VI) and that its ability to remain in solution is not surprising. Measurements of oxidation state ratios in Windscale effluent as well as at various distances from Windscale (and by inference at various times after release) are in progress. Nevertheless, the results of the present study have shown: (1) that plutonium can exist in natural seawater (at least in the one area for which data are available) as a mixture of

Table 1 Pu(IV) and Pu(VI) concentrations in water and suspended particulates

Sample location and type	Particle size* (μ m)	²³⁹ Pu(IV) (fCi l ⁻¹)§	²³⁹ Pu(VI) (fCi l ⁻¹)§	Pu(VI)/Pu(IV)
1 Shoreline†	>0.22	6,200 ± 300	600 ± 200	0.096 ± 0.032
	<0.22	300 ± 40	1,250 ± 60	4.2 ± 0.6
2 Shoreline†	>0.22	15,600 ± 500	610 ± 90	0.039 ± 0.006
	<0.22	70 ± 10	400 ± 30	5.7 ± 0.9
3 Shoreline†	>0.22	8,400 ± 600	280 ± 90	0.033 ± 0.011
	<0.22	60 ± 10	220 ± 30	3.7 ± 0.8
4 Shoreline‡	>0.22	15,900 ± 900	200 ± 70	0.013 ± 0.004
	0.025–0.22	8 ± 4	54 ± 28	—
	<0.025	66 ± 3	791 ± 20	12.0 ± 0.6
5 Surface‡	>0.22	111 ± 8	7 ± 14	0.063 ± 0.126
	0.025–0.22	1 ± 4	–8 ± 13	—
	<0.025	33 ± 3	242 ± 10	7.3 ± 0.7
5 Bottom‡	>0.22	1,250 ± 52	28 ± 17	0.022 ± 0.014
	0.025–0.22	–1 ± 6	–10 ± 8	—
	<0.025	83 ± 4	200 ± 6	2.4 ± 0.1
6 Surface‡	>0.22	524 ± 19	–22 ± 18	–0.042 ± 0.034
	0.025–0.22	78 ± 8	34 ± 19	—
	<0.025	49 ± 3	399 ± 13	8.1 ± 0.6
7 Surface‡	>0.22	57 ± 7	22 ± 42	0.39 ± 0.74
	0.025–0.22	5 ± 4	42 ± 18	—
	<0.025	54 ± 3	443 ± 13	8.2 ± 0.5

* As defined by filtration through Millipore membrane filters; this gives only a general indication of particle size.

† Samples collected 21 June 1977; subsample size = 0.8 l.

‡ Samples collected 10 August 1977; subsample size = 4.0 l.

§ Total of ²³⁹Pu + ²⁴⁰Pu; ±1 s.d. counting error.

Table 2 Distribution coefficients of Pu(IV) and Pu(VI)

Sample	Suspended load (mg l ⁻¹)	$K_d^{239}\text{Pu(IV)}^*$ (l kg ⁻¹ × 10 ⁻⁵)	$K_d^{239}\text{Pu(VI)}^*$ (l kg ⁻¹ × 10 ⁻⁵)	$K_d^{239}\text{Pu(total)}^*$ (l kg ⁻¹ × 10 ⁻⁵)
1	60	3.4 ± 0.5	0.08 ± 0.03	0.73 ± 0.05
2	110	20 ± 3	0.14 ± 0.02	3.1 ± 0.2
3	140	10 ± 2	0.09 ± 0.03	2.2 ± 0.3
4	40	60 ± 4	0.06 ± 0.02	4.7 ± 0.3
5 S	2	17 ± 2	0.14 ± 0.29	2.1 ± 0.3
5 B	5	30 ± 2	0.28 ± 0.17	9.0 ± 0.4
6	2	54 ± 4	-0.28 ± 0.23	5.6 ± 0.3
7	2	5.2 ± 0.1	0.25 ± 0.47	0.8 ± 0.4

* Total of ²³⁹Pu + ²⁴⁰Pu based on ± 1 s.d. counting error.

oxidation states; and (2) that it distributes between the soluble and particulate phases in a manner dependent on this mixture. It is clear that a knowledge of the changes in oxidation state undergone by plutonium will be important in understanding the ultimate fate of this element in the environment.

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Catastrophic events in a surface mixed layer

THE formation and evolution of mixed layers in the surface waters of the oceans are important in many fields of study and much effort has been expended towards the development of predictive models of the wind-mixed surface layer^{1,2}. These models parameterise small-scale high-frequency turbulent mixing processes, which are difficult to measure, in terms of more easily observed variables such as the wind stress. Because this parameterisation depends on hypotheses about the character of the turbulent mixing which dominates the transport of heat and

momentum, it is vital to have a realistic concept of the turbulent processes in active mixing layers. It is often assumed that a major source of turbulence at the base of the mixed layer is the shear between the mean currents in the mixed layer and those in the thermocline. Simple shear turbulence of this sort often may be described by a flux-gradient relationship³. We have now found that although a flux-gradient description is adequate most of the time at the base of an actively mixing layer, during a storm isolated and relatively rare large-scale catastrophic mixing events, which cannot be described by a flux-gradient relation, can dominate transport. Although these catastrophic events occur infrequently, the vertical heat transport associated with them can dominate the continuous transport associated with flux-gradient processes.

While taking microstructure profiles at Ocean Station P (50°N, 145°W) during August 1977, we observed a storm of substantial intensity (maximum wind speed 20 m s⁻¹) which lasted 48 h. Temperature profiles were taken from the RV Oceanographer with the microstructure profiler (MSP)⁴, a winged instrument which falls freely through the water measuring temperature, pressure, and conductivity. Sampling on 23 August began at 0415 GMT, about 24 h into the storm. During an 8-h sampling interval, 22 casts were made, each lasting about 5 min, with 10 to 60 min elapsing between casts. Since the vessel was by no means motionless during the storm, each cast must be considered as an observation of an independent realisation of the processes involved. No clear evidence of systematic mean temporal changes in the profiles can be seen during the sampling. Observation of mixed layer deepening during this period was obscured by 5-10 m amplitude internal waves.

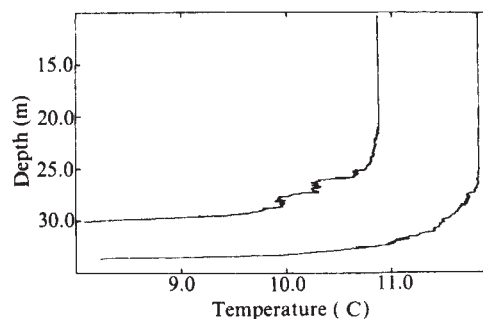


Fig. 1 Temperature profiles typical of small-scale, shear-generated turbulence at the mixed layer base. Left profile is offset 1 °C to the left. Thermocline depth difference is due to internal waves.

Most profiles on 23 August show the temperature to be nearly constant to within a few metres of the seasonal thermocline (Fig. 1). At the base of the mixing layer, a rather smooth transition from mixed layer to thermocline is usually seen; temperature structures seen here usually have a length scale less than several centimetres. This is characteristic of small-scale, shear-induced turbulence.

Two of the August 23 profiles are remarkably different (Fig. 2). They show events in which large masses of cool water are swept into the warmer isothermal layer above. In addition, two other profiles show large-scale disturbances at the mixed-layer base (Fig. 3). These may well be incipient events, in which the unstable mass of water has not yet separated from the water below.

Information regarding the vertical motion of these features can be obtained from the instantaneous fall rate of the probe. A winged vehicle with large drag coefficient responds rapidly to fluctuations in the local water velocity,⁵ and drops at a constant speed with respect to the water in its immediate vicinity. (If the probe's fall rate is inferred from the pressure record, the measured speed of descent is insensitive to vertical velocities associated with surface waves.) On encountering the sharp

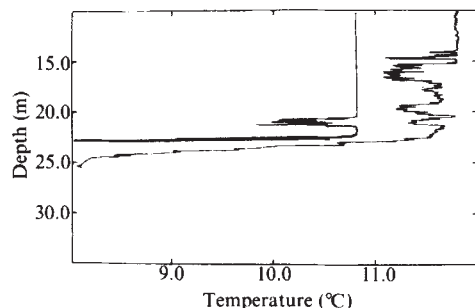


Fig. 2 Temperature profiles showing large-scale catastrophic mixing events. Event in left profile is very young, while event in right profile is older and in a state of collapse.

inversion in the profile on the left in Fig. 2, the probe abruptly ceased to fall relative to the surface, indicating that the water surrounding the probe was moving upwards at a speed comparable to the probe's vertical velocity, 12 cm s^{-1} . Thus, this water mass arrived from below only seconds before the observations, a conclusion borne out by the extremely large temperature gradients at its upper and lower edges. The event shown in the profile to the right in Fig. 2 was moving downwards at 5 cm s^{-1} , indicating that this water mass is older and in a state of collapse, consistent with the convoluted, somewhat diffuse character of its boundaries. The 'incipient' events in Fig. 3 each have an upward velocity of $2\text{--}3 \text{ cm s}^{-1}$.

The importance of the catastrophic events to mixed layer dynamics is revealed by a comparison with the more ordinary flux-gradient shear turbulence. An estimate of the heat flux at the base of the layer in the absence of large-scale mixing events is modelled as $H = -K_v \partial \theta / \partial z$ where K_v is estimated from the Cox number⁶ (the mean square temperature gradient normalised by the square of the mean gradient). In the absence of large events the heat flux at the base was of the order of $10^{-3} \text{ cal cm}^{-2} \text{ s}^{-1}$.

The heat flux attributable to catastrophic events can be described by the free-turbulence estimate $H = K_v \Delta \theta / l$, where K_v is estimated from Richardson's law of diffusion as $1/2 \epsilon^{1/3} l^{4/3}$, ϵ is the rate of dissipation of mechanical energy per unit mass, l is the size of the disturbance, and $\Delta \theta$ is the temperature difference between the mass and its surroundings. The heat flux is then $H = 1/2 \epsilon^{1/3} l^{1/3} \Delta \theta$, weakly dependent on ϵ and l and strongly dependent on $\Delta \theta$. The dissipation rate may be estimated from Fourier analysis of the temperature gradient by finding the Batchelor scale, the smallest length scale at which temperature fluctuations are appreciable. Lower bound estimates of ϵ , l and $\Delta \theta$ lead to a lower bound heat flux for a 'typical' event of $0.3 \text{ cal cm}^{-2} \text{ s}^{-1}$. Weighting the single-event heat flux by the probability of occurrence during the sampling period (0.1–0.2) yields a lower bound of $0.03 \text{ cal cm}^{-2} \text{ s}^{-1}$ for an average heat flux. Thus the flux-gradient heat flux is dominated by the heat flux due to catastrophic events despite their rarity.

The energy available for changing the potential energy of the water column by deepening the mixed layer is the difference

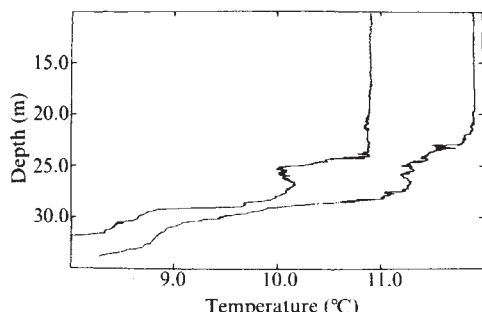


Fig. 3 Temperature profiles showing large-scale disturbance at mixed layer base which are interpreted as incipient catastrophic events.

between the energy produced by turbulence and the energy dissipated by viscosity. Most of the energy produced by simple shear-stress is dissipated; thus this process is inefficient in accomplishing mixed layer deepening. Large-scale mixing events can, however, cause large rapid transfers of kinetic energy directly from the mean flow through interaction with internal waves⁶, and thus be very efficient for mixed layer deepening. Instabilities in general are triggered when some dynamically important parameter, such as a Richardson number, reaches a threshold value. During a second milder storm on 31 August, apparently no threshold was reached, for no overturning events were found. We might therefore expect that realistic parameterisation of mixed layer dynamics in strong storms should include a threshold for the occurrence of catastrophic mixing events.

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²³⁰Th/²³⁴U age support of an interstadial sea level of -40 m at 30,000 yr BP

THE position of sea level about 30,000 years ago—in the Paudorf interstade in Europe¹ and the Plum Point interstade in northeastern America²—is disputed, estimates varying from above present sea level to $\sim 50 \text{ m}$ below it. The disagreement is due partly to ¹⁴C dating problems, and partly to stratigraphic errors and incorrect tectonic assumptions³. We now confirm a previous estimate^{4–6} of -40 to -42 m made from Huon Peninsula, New Guinea, and give new ²³⁰Th/²³⁴U data which support previous ¹⁴C results.

Raised coral terraces at Huon Peninsula contain more late Quaternary reefs than similar formations elsewhere (such as in Barbados⁷ and Timor⁸) because of higher uplift rates, and are the basis for the palaeo-sea level curve for the last 0.25 Myr given in ref. 9. We are concerned with Huon terrace II dated previously by ¹⁴C at $28.5 \pm 0.6 \text{ kyr}$ (mean of two determinations⁵ with seawater ¹⁴C correction¹⁰ subtracted). The ¹⁴C dates are from massive clams, *Tridacna gigas*, which show very little inversion from aragonite to calcite and which are microscopically indistinguishable from *Tridacna* specimens known to be beyond the ¹⁴C dating limit and which are measured as containing zero ¹⁴C—hence the result of 28.5 kyr has been argued as being a satisfactory ¹⁴C age estimate⁵. However, because of ¹⁴C dating uncertainty for old shell samples reservations have been held about this result, in the absence of confirmatory ²³⁰Th/²³⁴U measurements⁶.

A specimen of unaltered coral, *Favia speciosa*, collected from the crest of Huon reef II and Blucher Point ($147^\circ 40' \text{E}$, $6^\circ 10' \text{S}$), 18 m above sea level, yields the following U-series results: U-content = 2.62 p.p.m. , ²³⁴U/²³⁸U = 1.2 ± 0.02 , ²³⁰Th/²³⁴U = 0.25 ± 0.02 , age estimate = $31.0 \pm 2.5 \text{ kyr}$. We believe that this

reef crest is time-equivalent with that from which the ^{14}C dates were obtained (at $147^\circ 37'\text{E}$, $6^\circ 7'\text{S}$), based on continuous reef-tracing between the two sites. Analytic methods are explained in ref. 8.

We consider that these results confirm an age-estimate of 28.5 to 31.0 kyr for Huon reef II. In the context of rapidly rising Huon Peninsula, the associated palaeo-sea level must be estimated by subtracting (age $\times$ uplift rate) from present elevation of reef II. For this, Chappell⁵ used a nonlinear uplift rate model; Bloom *et al.*⁶ used a linear model. Reef II results differ little between these two models and indicate a palaeo-sea level of -40 to -42 m. The structure of reef II indicates sea level rise before growth of the dated crest; a subsequent fall to about -140 to -150 m before post-glacial transgression has been interpreted from young submerged terraces and reefs⁵. A low sea level around -150 to -160 m, relative to this part of the globe about 17,000 years ago, is supported by dated submerged terraces off northern Australia^{11,12}.

These sea-level changes conflict with ice volume variations estimated from ^{18}O analysis of deep sea cores. Figure 1 compares Huon Peninsula sea levels for the past 45 kyr (refs 8, 9), with ^{18}O curves from Panama Basin core V19-28 (ref. 13) and Caribbean core P6304-9 (ref. 14) using the chronology of Shackleton and Opdyke¹⁵ and Shackleton and Matthews¹⁶. Also shown are ^{14}C -dated variations of the southern margin of the Wisconsinan ice sheet². Correlation of Huon Peninsula sea levels seems to be better with the ice margin changes than with the ^{18}O records. Note, however, that the ice margin curve is not zero-referenced, because the parameter discussed here is ice volume, which is very difficult to estimate before 17 kyr because of uncertainty about previous ice margin patterns^{2,17}.

Fig. 1 Sea levels relative to Huon Peninsula, $\delta^{18}\text{O}$ variations in Caribbean and central Pacific surface waters, and migration of southern margins of Laurentide ice sheet. Note that deep-sea core chronologies are based on interpolation¹⁵ and may not strictly be comparable with the sea level and ice margin chronologies.

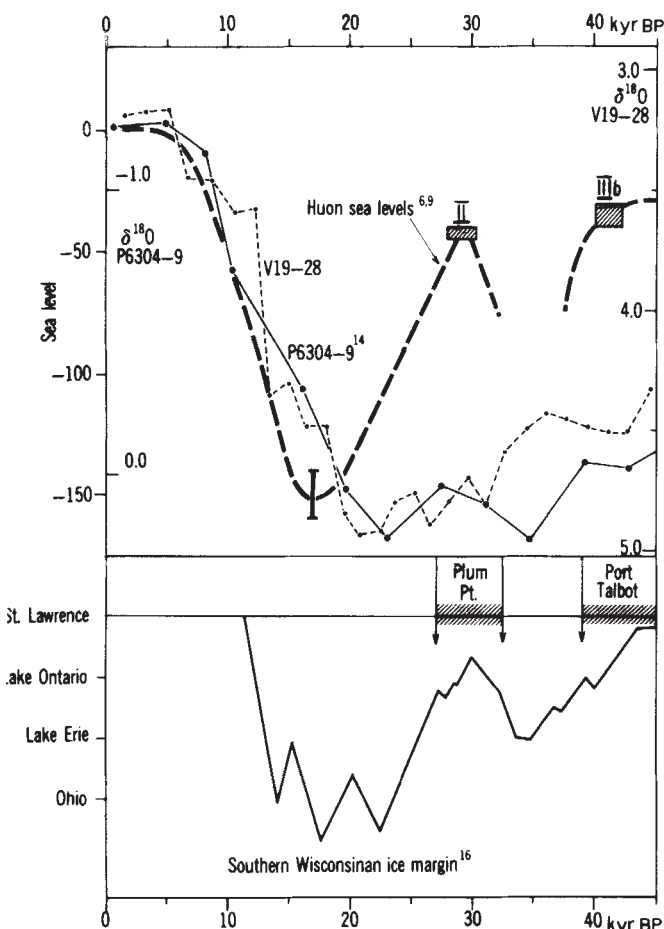


Table 1 Comparison of $^{230}\text{Th}/^{234}\text{U}$ and ^{14}C ages

Site	Ref.	C age (kyr)	No. of determinations	Th/U age (kyr)	No. of determinations
Huon P.	10	6.76	4	5.8	2
Huon P.	10	7.64	4	9.4	4
Gt Barrier Reef	11	13.7 ± 0.2	2	17.0 ± 0.8	2
Huon P.	This work	28.5 ± 0.6	2	31.0 ± 2.5	1
Huon P.	5, 6	35.6 ± 1.5	2	40 ± 3.0	4

Two factors damp the ^{18}O ice-volume signal in deep sea cores—seabed mixing processes, and variation of average ^{18}O content of the icecaps themselves⁹. However, the discrepancy for reef II, as well as for reef IIb (ref. 5) (Fig. 1), is so large that one of the following three conditions must apply:

(1) Sea level relative to Papua New Guinea and northern Australia was no lower than about -75 m instead of the -150 m interpreted from dated submarine reefal terraces.

(2) A non-eustatic factor has affected the Huon Peninsula sea-level curve—either highly nonlinear tectonics, or hydro-isostatic processes greater than previously calculated^{18,19}, or some other process such as geoid changes²⁰.

(3) The $\delta^{18}\text{O}$ records are significantly smoothed by seabed mixing processes, reducing amplitude of peaks of less than ~ 10 kyr. Cores with higher sedimentation rates such as V19-28 show more variation than lower rate cores (compare V28-238)¹⁵, and cores with yet higher rates may well offer a good test of our sea level curve.

Finally, we wish to comment on ^{14}C and $^{230}\text{Th}/^{234}\text{U}$ chronologies. Table 1 lists comparative data from several reefs—many from the same specimens—from ~ 6 to 45 kyr, $^{230}\text{Th}/^{234}\text{U}$ results beyond 6 kyr consistently show greater ages than ^{14}C . In the Holocene range the ^{14}C ages are more 'accurate' in terms of geochemical equivocations; however, they are well known to appear younger than true age due to past variation of atmospheric ^{14}C production. Concerning older examples in Table 1, corroboration seems to come from recent thermoluminescence dates from archeological materials ~ 30 kyr old, which like our reef II results, appear similar to ^{14}C dates from the same sites^{21,22}. The problem of minor ^{14}C contamination in samples beyond ~ 30 kyr is difficult to eliminate, although we note that our $^{230}\text{Th}/^{234}\text{U}$ age for reef II is very close to the ^{14}C age of the Plum Point Interstade climax (Fig. 1), suggesting that the two chronologies become close at that time.

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Eastwards migration of the Tuscan anatectic magmatism due to anticlockwise rotation of the Apennines

AT the northern apex of the Tyrrhenian Sea, two mountain chains face each other displaying opposite vergence: the Corsican alpine chain and the Northern Apennines (Fig. 1). The Corsican alpine chain consists of both oceanic and continental nappes transported towards the West which are assumed to have derived from the peeling off of European, Tethyan and African (Austroalpine domains) lithosphere sectors. The age of metamorphism and orogenic transport towards the European foreland is mainly Cretaceous-Palaeogene. We present here an interpretation of the available data on post-collision continental anatectic magmatism in the Tuscan province (Italy) in the light of geodynamic evolution of the Tyrrhenian Sea.

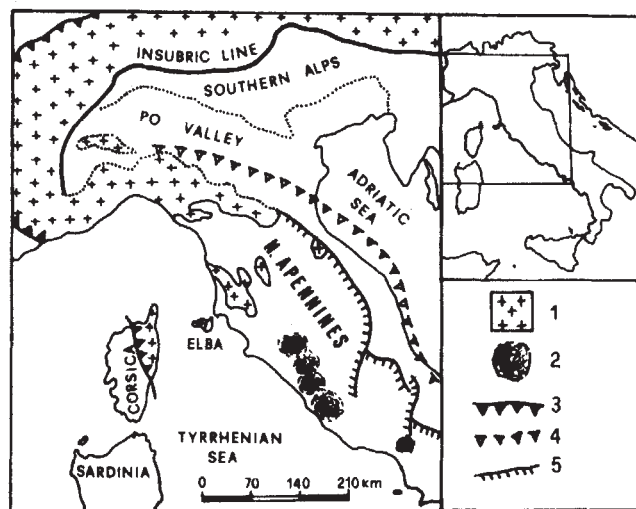


Fig. 1 Tectonic sketch map of northern Italy. 1, Palaeogene and Neogene Europe-verging Alpine nappes; 2, main upper Tertiary-Quaternary volcanoes; 3, European front of the Alpine chain; 4, front of the Apennines; 5, front of the carbonate units in the northern and central Apennines.

The northern Apennines consist of east and northeastwards transported continental nappes peeled off from the African (Insubric-Apulian domains) lithosphere sector. Here, the age of metamorphism and orogenic transport is mainly Neogene. The Ligurian nappes, considered to represent slices of oceanic crust¹, are interpreted here as part of the Cretaceous-Palaeogene Europe-verging Alpine chain. During the late Oligocene and early Miocene they partially overthrust as a whole on to the Insubric domains that in Neogene times formed the Africa-verging Apennine chain (Fig. 2).

Extensive magmatic activity (the Tuscan province) developed between Corsica and Tuscany, within the strongly deformed belt marking the collision zone between the two chains. The crustal anatectic origin of this magmatic province is indicated both by petrological evidence (xenocrysts of quartz, cordierite and sometimes sillimanite, corundum normative composition² and isotopic data (high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios^{3,4}, high $\delta^{18}\text{O}$ (ref. 5)). Absolute age determinations range from 8.5 on Elba Island in the west to 0.43 Myr in Mt Amiata in the east^{4,6} (Fig. 3).

The deep crustal structure in this area is controversial. The interpretation of gravimetric data⁷ suggests crustal thicknesses

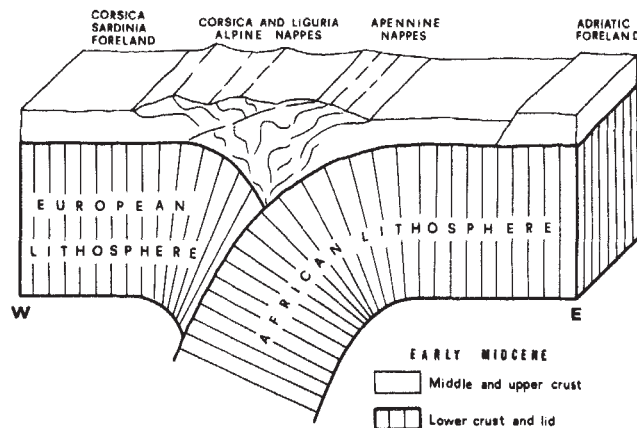


Fig. 2 Palinspastic restoration of the Corsica-Sardinia and Apulia margins at the end of Palaeogene.

under western Tuscany of ~25 km. This value is too small for the root zone of a Neogene mountain chain, which implies that the crust here has been thinned and rifted after continental collision. A new interpretation has been proposed based on seismic results and the best fitting of seismic, gravimetric and magnetic data^{8,9}: according to this model, the area between Elba and western Tuscany is characterised by the existence of two crust-mantle boundaries, the first 15-25 km deep and the second 45-50 km deep. The body between the two discontinuities might contain continental crustal material, a possible source for the observed magmatism. Accepting crustal thicknesses around 45-50 km the western Tuscany area might correspond to a normal or slightly thinned root zone for the northern Apennines.

Figure 3 shows all the published geochronological data available concerning the Tuscan province; new K/Ar age determinations on a peralkaline lamprophyre outcropping near Sisco (northeastern Corsica) are added here.

Velde¹⁰ first defined the potassic peralkaline nature of the Sisco lamprophyres. The mineralogy is phlogopite, sanidine, olivine, quartz and an uncommon amphibole (potassic richterite). All the data strongly support the geochemical affinity of these rocks with those of the Tuscan province¹¹. The dated samples were collected from the outcrop described and analysed by Velde¹⁰ and belong to a sill intruded within the ophiolitic nappes (Schistes lustrés). Whole rock and separated phases were dated by the K/Ar method (Table 1). The errors (1σ) have been evaluated using the Cox and Dalrymple formula¹². The K/Ar dates range from 14.3 to 15.4 Myr. In a plot of $^{40}\text{Ar}/^{36}\text{Ar}$ against $^{40}\text{K}/^{36}\text{Ar}$ they define an isochron of 14.2 ± 0.2 Myr with an initial $^{40}\text{Ar}/^{36}\text{Ar}$ value of 300.1 ± 0.6 . This age is the oldest so far

Fig. 3 Simplified map of the Tertiary and Quaternary magmatic activity in the Tuscan province. Figures indicate the ages (Myr).

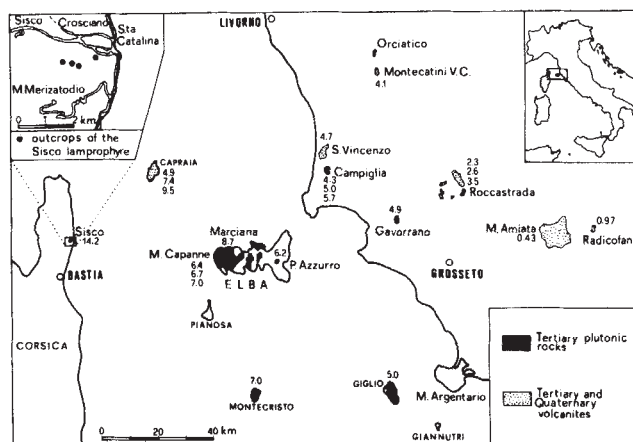


Table 1 K–Ar ages of the Sisco peralkaline lamprophire*

Analysed phase	K (%)	^{40}Ar rad (%)	^{40}Ar rad ($\times 10^{-10} \text{ mol g}^{-1}$)	$^{40}\text{Ar}/^{36}\text{Ar}$	$^{40}\text{K}/^{36}\text{Ar}$ ($\times 10^3$)	Age (Myr)
Whole rock	7.15	57.6	1.82	696.3	481	14.3 (± 0.4)
K-Feldspars	8.86	32.1	2.44	430.1	152	15.4 (± 0.5)
Phlogopite (60–100 mesh)	8.00	21.0	2.06	373.9	93.4	14.4 (± 0.5)
	8.00	46.7	2.10	551.1	298	14.7 (± 0.4)
Phlogopite (100–140 mesh)	8.11	21.1	2.23	372.9	87.3	15.3 (± 0.5)
	8.11	27.9	2.13	409.0	132	14.7 (± 0.5)

* ^{40}Ar was determined by isotope dilution mass spectrometry, using a GD-150 Varian Mat mass spectrometer and the ^{38}Ar -enriched Zurich spike. The calibration was checked by LP-6 USGS standard, a muscovite internal standard (MCT) and Bern LP-6 biotite. K was determined in duplicate by flame atomic absorption with a Perkin Elmer 303.

* $\lambda_e = 0.585 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K}/\text{K} = 1.19 \times 10^{-4} \text{ atom/atom}$.

found in the north-Tyrrhenian post-Palaeogene magmatic province. Figure 4 shows these new data, and literature values, in an age against distance plot; the distances have been computed by projecting the magmatic centres on the Bastia–Radiocofani line. The new data from North Corsica confirm the eastwards migration of the magmatism^{4,6} giving 1.3 cm yr^{-1} as a mean migration velocity.

Contrasting models have been proposed to explain this age–space relationship. Alvarez¹³ argued that the Tuscan magmatic activity belongs to a calc-alkaline sequence related to a former Benioff plane dipping eastwards under Italy. According to the Alvarez model the eastwards migration of the magmatism results from the subduction and simultaneous anticlockwise rotation of the Corsica–Sardinia microplate. Marinelli¹¹ postulated the existence of a hot spot under Tuscany ('Etruscan swell') and suggested that the eastwards migration of the magmatic activity is evidence of a movement of the Tuscan continental lithosphere towards the west with a velocity of about 1 cm yr^{-1} .

Our new model might explain better the genesis and the eastwards migration of the Tuscan magmatic activity, taking into account the main geodynamic events occurring in the Tyrrhenian area during Neogene and Pleistocene times.

The beginning of the Tuscan anatectic magmatism overlaps with the final phases of the Sardinian calc-alkaline volcanism¹⁴. This volcanism has been recently associated with the subduction of the continental Insubric–Apulian lithosphere dipping westwards beneath the Corsica–Sardinia block (Iberia plate)¹⁵. The Oligocene and Neogene Africa-verging nappes of the Apennines are interpreted, in this scheme, as peels detached from this subducting lithosphere and pushed toward the east and north-east¹⁵ (Fig. 2).

Sialic material packed in the roots of the Apennine chain during the main compression phases might represent the source of the anatectic magmas. At that time, before the middle Miocene, the Tyrrhenian Sea had not yet developed as an extensional basin. Starting from middle–late Miocene times, tensional tectonics characterised the Tyrrhenian area, until an oceanic plain had developed by Messinian times^{16,17}. The Tyrrhenian seafloor spreading is supposedly connected with an anticlockwise rotation of the Apennines¹⁵. According to this model, and assuming a rotation pole in the Tuscan area or further north, it is possible to reconcile the simultaneous continental magmatic activity in Tuscany with the tholeiitic activity on the Tyrrhenian abyssal plain during late Miocene and Pleistocene times.

The generation and ascent of the magma is probably a result of heat flow increase, pressure relief and fluid mobilisation during a period of tensional rift tectonics. The heat flow increase could be due to a hot spot¹¹ and/or a general rise of the isotherms in response to thinning of the lithosphere during rotation of the Italian peninsula. In any case, the considerable radioactive heat generation of the upper crustal material in the root zone should be taken into consideration. Furthermore isotopic and geochemical data reported for the Tuscan magmatic rocks support this model and suggest a mixing of upper crustal materials^{2,18} with mantle derived magmas.

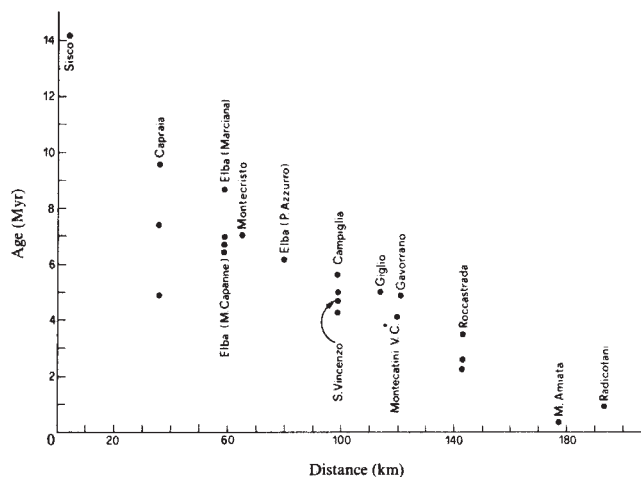


Fig. 4 Ages versus the distances of the magmatic episodes in the Tuscan province. The distances have been computed by projecting the magmatic centres on the Bastia–Radiocofani line.

The eastwards migration of the magmatism can be easily related to a progressive crustal thinning and subsequent rifting due to the anticlockwise rotation of the Apennines. The Tyrrhenian abyssal plain, being far from the rotation pole, is the area in which tensional tectonics are most apparent, so that thinning and rifting of the continental crust was followed by the generation of new oceanic crust in the south.

Finally, such a rotation model may explain the simultaneous late Miocene–Pliocene longitudinal rifting of the chain along the Tyrrhenian border and the nappe and fold development along the Adriatic front.

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Do aerosol anomalies precede earthquakes?

REPORTS of strange animal behaviour in my home village, which was struck by the Friuli earthquake in northeastern Italy (6 May 1976) convinced me that aerosol particles were electrostatically charged before the quake and that the physiological effects were essentially caused by an increase of the ion concentration in the air resulting in reactions such as the serotonin irritation syndrome. A mechanism is suggested here for the liberation of electrical charge from underground: electrochemical glow discharge.

The most remarkable animal behaviour effects (the quake occurred at 2100 LT) were the following (a more detailed description is given elsewhere¹). Deer formed flocks—in the late afternoon, a flock of 15 deer came down from the mountains and close to the village, crowding together with apparently no interest in grazing—an event never before seen in this area. Cats left the houses and the village—at the time of the quake no cat was apparently left in the village. They did not return until two days later (there were many aftershocks). In three cases, cats dragged kittens outdoors and bedded them in green vegetation. Before fleeing from an apartment a cat was seen to move its ears convulsively for some time. Mice and rats left their hiding places—on one farm mice and rats were observed running around before the quake. People were annoyed and surprised as all their five cats were missing. Fowl refused to roost a few hours before the quake. People fleeing from their houses during the quake found their fowl already scattered in the garden. The animals had apparently forced their way out from the pen before the disaster. On one farm, several minutes before the quake, the fowl made such a noise that farmers thought a fox had entered the chicken house. Cattle panicked in their barns—according to many reports, cattle showed clear signs of fear 15–20 minutes before the quake. The animals started to bellow, tear at their chains and paw their boxes. Dogs barked without apparent reason. This behaviour started 20 min before the quake. Some people were guarding their property, suspecting intruders, just before the quake hit. Birds emitted calls at unusual times—one person heard a cuckoo, never normally heard at night. Caged birds started to fly—in six places people observed that 10–15 min before the earthquake birds became restless and occasionally emitted calls. Then they began to beat their wings and finally started to fly. A bird kept its plumage wet—in the late afternoon before the quake occurred, one woman observed that her canary had emptied the water bowl which was filled daily and generally only half emptied in one day, she refilled it and shortly before the earthquake found it empty again; the bird must have kept wetting its plumage.

The characteristic animal behaviour observed in northeastern Italy by people with little experience of earthquakes (independent accounts from neighbouring areas are published elsewhere²) is essentially identical to that reported from China^{3–7}. This can be seen from a publication by the Earthquake Office in Tientsin⁸. It advises that an earthquake may be imminent when cattle, sheep or horses refuse to get into the corral, when rats run out from their hiding place, when chickens fly up to the trees and

pigs break out from their pens, when ducks refuse to go to the water and dogs bark for no obvious reason, when snakes come out from the winter hibernation, when pigeons are frightened and will not return to their nests, when rabbits with their ears erect jump up and crash into things and when fish jump out of the water as if frightened.

There are many other accounts of anomalous animal behaviour before earthquakes (see refs 9–21): 33 independent reports from all over the world were collected by the US Department of the Interior¹⁸ and I have collected 78 reports with abundant supporting evidence from folk tradition and mythology¹. After the successful prediction of the earthquake of Haicheng in China in 1975 the dramatic increase in the number of reports of anomalous animal behaviour was quoted as one of the principal reasons, as well as foreshock activity and water level changes, for the declaration of the state of emergency.

In the search for scientifically useful clues to the phenomenon I studied the case of a livestock train standing in a small town (Pontebba) awaiting customs clearance on the evening of the earthquake. The wagons were boxes of sheet iron with several rectangular openings only. The cattle and horses had been fed and watered. Under normal circumstances they stay quiet. Approximately 15–20 minutes before the earthquake, however, the animals started to show discomfort and produce excessive noise. The transportation workers decided to notify their superiors, fearing poisoning or disease. These wagons (Faraday cages) cannot apparently shield these pre-earthquake signals. Nor could small vibrations preceding the quake have been responsible for the unrest. There was only one, relatively strong, foreshock within one minute of the main quake and how could animals that have been travelling by train for days suddenly get panic-stricken? Why are birds that live in the trees frightened and how can such hypothetical small vibrations be distinguished from similar ones produced by trucks and trains?

The clue to this mysterious animal behaviour was finally found, in my opinion, when I spoke to a retired precision mechanic. As the time of the earthquake approached he was repairing a small wrist-watch. One operation consisted in placing a thin lamella of stainless steel—estimated to weigh a few tenths of a gram—on the clockwork. It would not stay there but jumped from its place. After several futile attempts the confused man went and looked at the weather outside; the sky was completely cloudless. After resuming his work he still found the strong electrostatic repulsion phenomenon; immediately afterwards the first shock occurred.

Only charged aerosol particles could have caused such a strong charging of metal parts in the same polarity. It led me to hypothesise that before the earthquake an unknown geophysical phenomenon liberates electrostatic charges from underground.

The Menlo Park Conference on abnormal animal behaviour before earthquakes¹⁸ gave this hypothesis some additional weight by advancing arguments against several alternative potential geophysical signals of non-electric origin. Underground noise would not be heard better by most domestic animals, as the conduction of ultrasound signals to which they are more sensitive is drastically attenuated during its passage through rocks. Changes of the magnetic field of the Earth as well as possible barometric pressure changes would be smaller than those variations which most animals would usually experience and microshocks would also be detected by seismographs apart from being too frequent to cause serious discomfort to animals.

This hypothesis is supported by much circumstantial evidence¹ among which the following is the most convincing.

(1) There are many reports of physiological effects of charged aerosol particles on animals and humans²². Much work has been done on the action of small air ions (as the smallest charged aerosol particles are usually called), especially by A. P. Krüger *et al.*^{23,24}, who succeeded in tracing the biochemical factor responsible for the action of air ions on living beings. Experiments have shown that small positive ions increase the production of serotonin, a powerful neurohormone, engaged in physiological functions such as sleep, moods and the transmission of nervous

impulses. Subsequent investigations²⁵⁻²⁸ supported these results and revealed that a typical kind of illness, felt during certain weather situations such as the Föhn in the Alps or the Sharav in the Near East, which are also characterised by abnormally high concentrations of positive ions, is also accompanied by a high serotonin production. The cluster of symptoms (migraine, nausea, vomiting, irritability, hyperperistaltis) was called the serotonin irritation syndrome. It can be successfully treated with negative ions or serotonin blocking agents.

(2) An electrostatic charging of aerosol particles can produce fog in air with less than 100% humidity²⁹. Fogs or strange clouds have been explicitly designated as earthquake precursors since the days of Aristotle and Pliny, and there is a long record of such observations in Europe¹⁰, America¹, and Japan¹⁴ (chiki = the air from the ground). A detailed description of such a phenomenon is, for example, given by A. von Humboldt for an earthquake in Cumana, Venezuela¹⁹.

(3) Light-producing electrical discharges are to be expected as a consequence of strong charging of aerosol particles. There are many reports of luminous phenomena during and before earthquakes.^{9,14,19,20,30-33}

(4) The proposed electrical nature of the short-term earthquake precursory phenomenon makes it qualitatively comparable with transient electrical phenomena in the atmosphere preceding dramatic weather changes. Many characteristic features of reported animal behaviour are qualitatively similar before major storms¹. It is possible that animals see in an earthquake a phantom storm. Their reactions might therefore partly be due to a weather instinct which is distorted by the unusual intensity in the development of the event and the physiological effect of air ions. This would explain the evolution of an 'earthquake sense' in animals in the absence of appropriate opportunities for natural selection.

(5) The atmospheric changes preceding strong earthquakes are apparently also felt by sensitive people. The term 'earthquake weather' is known in Europe, South America and in western US¹. It is associated with an unusually warm and oppressive weather situation. The reported physiological reactions⁹ are comparable to those felt during Föhn, Scirocco or Sharav, which are known to have been accompanied by high concentrations of positive air ions.

(6) There are old reports of qualitative measurements of the electricity of the air by means of electrometers during periods of aftershocks. One account is by A. von Humboldt¹⁹ from Venezuela (1799) and one by V.-Eandi from Piemont^{34,35} (1808). Both scientists report a marked increase of electricity during shocks. For both earthquake events there are parallel reports of anomalous animal behaviour. In certain descriptions of anomalous animal behaviour the electrostatic nature of the underlying phenomenon becomes directly evident. According to de Ballore³⁶, in Sicily it was said that an earthquake was imminent "when cats start crying, bristle their hair and sparks are released from their backs". Additional evidence is provided by the Chinese experience⁶, that animals of very different temperament and sensitivity (58 species) are aware of approaching earthquakes. Remarkably, smaller animals (larger surface-to-volume ratio) are generally found to be more sensitive than bigger ones.

(7) The charged aerosol hypothesis does not only give an apparently consistent explanation of all important earthquake precursory phenomena from the popular tradition, which are occurring in the atmosphere, but is also consistent with an essential feature of the main earthquake theory of antiquity as elaborated by generations of Greek philosophers and especially by Aristotle. This claims that, before an earthquake, a strange gas (apparently warm and viscous) the 'pneuma' evolves from the Earth producing a series of atmospheric earthquake precursor phenomena (and the earthquake itself). The 'pneuma' of Greek philosophers could have been electricity of the air.

It is quantitatively justifiable to consider the piezoelectric effect in quartz-containing rocks (the average concentration of quartz in the Earth's surface is 15%) as the primary source of the

electrostatic energy. There is reportedly sufficient alignment of piezoelectric microcrystals to produce macroscopic effects³⁷ and Finkelstein and Powell have estimated that many thousands of volts per metre could be generated by pressure waves of earthquakes (25–250 bar) (refs 38, 39). Attempts to explain earthquake lightning as a consequence of macroscopical electrical discharges through the air, however, failed to be convincing because of the measured low resistance of the ground ($10^3 \Omega\text{m}$) compared with that of the air ($10^9 \Omega\text{m}$). Electrical currents in the ground would rapidly neutralise separated charges.

These currents could, however, be the ultimate cause of charge liberation by inducing a phenomenon known as electrochemical glow discharge or glow discharge electrolysis^{41,42}. The discharge is easily produced if the anode of an electrochemical cell is lifted slightly above the electrolyte's surface and the applied voltage simultaneously increased to maintain the electrical current across the gap. The resulting glow discharge can be maintained with a potential difference as low as 500 V and, if the gap is sufficiently thin even at atmospheric pressure (contact glow discharge⁴³). Positive ions (mostly H_2O^+), accelerated towards the water surface will generate radicals and ions with yields comparable to that of α -particles. But attainable dose rates with mA-glow discharge currents can exceed that of conventional radiochemistry by a factor of 100–10,000 (ref. 42). Microscopic electrochemical glow discharges resulting from piezoelectric transient phenomena and the passage of electrical current across tiny fractures in rocks and thin water films also seem to provide a quantitatively satisfactory mechanism. Glow discharges in the range of 1 mA (telluric currents of this order of magnitude have been shown to occur in connection with earthquakes and volcanic eruptions^{5,40}), with 3.7×10^{17} ions min^{-1} accelerated across 100 V towards a water surface could drastically change a typical ion concentration in the range of 10^4 – 10^6 cm^{-3} in the air above.

My consideration of many very old or inexact observations to support the proposed hypothesis does not meet present-day scientific standards and might provoke criticism. I believe this is unjustified as long as corresponding modern investigations do not exist. The question also arises as to whether our exclusive reliance on instrumental research (with the exception of China) and renunciation of a vast treasure of observations by scientifically unqualified people is justifiable in the case of earthquakes. In support of this view a remarkable fact should be mentioned: 1,900 yr ago the Roman naturalist and writer Pliny the Elder specified four different signals, which could indicate a threatening earthquake⁴⁴. Three of these, the appearance of foreshocks, turbidity in water wells and frightened birds, are considered relevant today, as they were used to forecast the earthquake of Haicheng in China^{3,5}. The fourth, the appearance of an atmospheric sign, a special kind of fog in an otherwise clear sky, could prove to be relevant if the hypothesis proposed in this paper were confirmed. Do we know more about short-term earthquake prediction than did the Romans?

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Evidence for domestication of the dog 12,000 years ago in the Natufian of Israel

THREE canid finds from the Natufian in the northern Israeli sites of Ein Mallaha (Eynan) and Hayonim terrace indicate a special man-animal relationship. These consist of a diminutive carnassial and mandible, and a wolf or dog puppy skeleton buried with a human. The finding of a puppy skeleton in such close association with man is of particular significance as an indication of a close relationship between man and dog.

A domestic animal is one whose breeding is largely controlled by man. Evolution of a domesticated species, therefore, results mainly from artificial selection, with natural selection playing a subsidiary part. The process of domestication implies the separating off of a breeding stock from its wild forebears. Most workers agree that the wild progenitor of the dog was the wolf *Canis lupus*¹⁻⁵.

A *Canis* skull from the Mesolithic site of Star Carr, England (9,538 ± 350 BP) was identified as a dog, on the basis of dental overlap². Lawrence³ identified dog from Idaho (USA, 10,400 BP) and from eastern Anatolia (~9,000 BP). More recently, Turnbull and Reed⁶ suggested that a mandible from the Zarzian (~12,000 BP) of Palegawra cave (Zagros mountains, Iraq) belonged to a dog, on the basis of dental overlap and small size.

The finds we report here come from the Natufian, an Epipalaeolithic culture (formerly referred to as a Mesolithic culture) and dates to 12,000–10,000 yr ago. The Natufians, whose culture was limited to the Levant, are considered to have been hunter-gatherers, and were the first peoples to live in circular dwellings in what were perhaps the earliest permanently settled villages. Their sites, compared with those of preceding periods are larger, occupying areas of 1,000 to 3,000 m², and contain numerous pounding stones (pestles and mortars) as well as, for the first time, large cemeteries. Art forms are also found, as is a highly sophisticated tool kit, consisting largely of micro-

liths, including lunates. They were the antecedents of the first agriculturalists in the region.

Bate's claim⁷ for the domestic status of a *Canis* skull from the Natufian of El Wad cave (Mount Carmel, Israel) could not be confirmed⁸. Recent excavations at two Natufian sites in northern Israel, Mallaha (Eynan) and Hayonim terrace, provide what we believe to be more concrete evidence for some kind of special relationship, perhaps domestication, between man and dog/wolf in this early period, around 12,000 BP.

The site of Mallaha, near the old Huleh lake in the upper Jordan valley, has been under excavation since 1955 (ref. 9). Two phases of Natufian occupation have been distinguished¹⁰: the canid osteological remains, a mandible from a living floor and a puppy from a tomb, both come from the lower one. This has so far revealed three superposed half-buried dwellings, originally probably circular and 9–11 m in diameter, whose northern halves are missing. The walls, some preserved to a height of more than 1 m, were built of limestone blocks, piled more or less vertically. The flint industry is characterised by lunates rarely less than 1.5 cm in length, most of which bear 'Helwan retouch', indicating an Early Natufian assemblage. Basalt pestles and mortars are fairly frequent, as are bone points, needles, pendants and some bone sickle handles.



Fig. 1 Tomb H. 104 at Mallaha, showing the human skeleton and puppy.

In the entrance to dwelling 131, the oldest so far excavated in Mallaha, a large slab of limestone was found on the floor. This has been interpreted to indicate the presence of a burial (H. 104, ref. 11) especially due to its proximity to a human skeleton which lay 25 cm below it. The human skeleton, whose sex cannot be determined because of the damage of the pelvis, lay flexed on its right side, and judging by the state of its dentition, was an old individual. Its left wrist was partially under its forehead, and hand upon the thorax of a puppy, which has evidently been buried complete with the human (Fig. 1). All the milk teeth of the puppy are fully erupted, and of the long bones exposed, a humerus has both epiphyses open, indicating that the age at

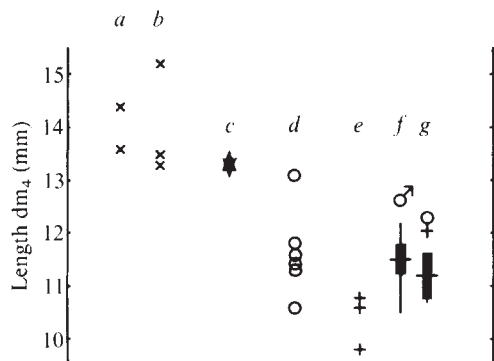
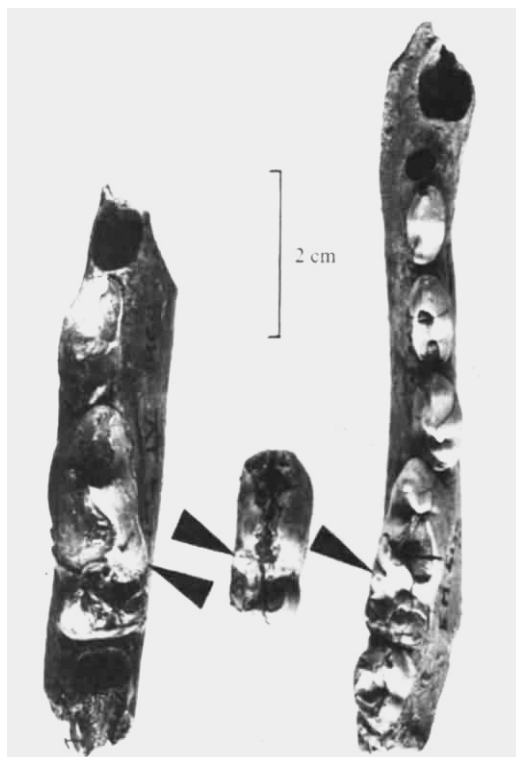


Fig. 2 Maximum length (mm) of the last lower milk molar (dm_4 , milk carnassial) of canid puppies. *a*, Two wolves from Turkey; *b*, three wolves from Israel; *c*, the Mallaha puppy; *d*, six dogs from Turkey, Israel and Egypt; *e*, three jackals from Turkey; *f*, 14 male jackals from Israel, range, mean $\pm$ 95% confidence limits; *g*, six female jackals from Israel, range, mean $\pm$ 95% confidence limits. (Data from Turkey supplied by S. Payne).

death was between 3–5 months¹², while the advanced state of wear of the lower milk carnassial makes an age of 4–5 months more likely. The upper teeth are too damaged to be measured, but the dm_4 which is intact, has a maximum crown length of 13.3 mm. Comparison with the same tooth of recent Israeli and Turkish wolves, jackals and dogs (Fig. 2) indicates that the fossil puppy was either a dog or a wolf, but not a jackal, being outside the size range of the latter.

An adult canid mandible was found in dwelling 131. It came from an undisturbed living floor, characterised by seven post holes, and lay not far from two hearths containing bones which included a human cranium and a pair of gazelle horn cores. The mandible is complete from canine to second molar and belongs to a large canid. The metaconid on the M_1 is poorly developed (Fig. 3) as in wolf and dog; unlike the jackal metaconid which protrudes lingually and is more prominent^{1,13}.

Fig. 3 Occlusal views of the Hayonim terrace carnassial (centre) and Mallaha mandible (right) compared with an Upper Pleistocene wolf mandible fragment (P_4 and M_1) from Ein Gev IV (Jordan valley, Geometric Kebaran A period). The M_1 metaconids are arrowed.



Osteological separation of the dog and wolf is problematical, but tooth crowding and smaller size have been used as criteria for the identification of dogs. It has frequently been claimed that dog jaws are slightly shorter than those of their wolf progenitors, resulting in a certain degree of dental overlap, particularly of the lower fourth premolar and the lower first molar^{2,3,6}. This dental overlap was analysed using an index of overlap (crown length of $M_1 \div$ alveolar length from P_4 to M_1). The value for the Mallaha mandible is 0.67, indicating some degree of overlap (Fig. 3). A collection of nine recent dogs from Israel and Egypt, however, gave values from 0.65 to 0.70 (mean 0.68) and a collection of 34 recent wolves from Israel gave values from 0.62 to 0.67 (mean 0.65), with six specimens actually possessing values of 0.67. Another index, the length $M_1 \div$ alveolar length of the lower premolars, showed a similar overlap between dogs and wolves. These indicate that dental overlap criteria are not very efficacious in dog–wolf separation, and are probably to some extent age dependent.

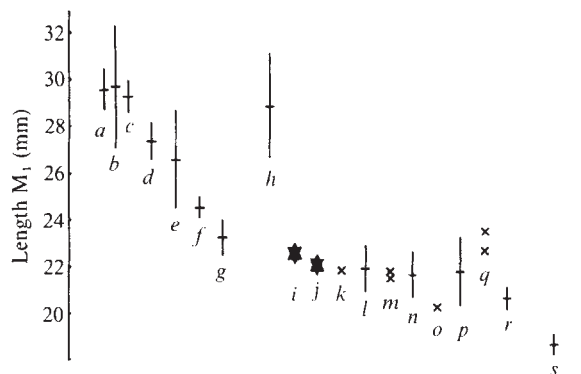


Fig. 4 Antero-posterior length of M_1 of recent and fossil canids. These show the north–south size cline in recent palaeartic wolves (*a* to *g*); the Pleistocene–Holocene diminution in the Levant (*h* versus *f*) and the wolf–dog differential (*f* versus *i* to *r*). Samples are represented by their mean $\pm$ 95% confidence limits, single specimens by a star or cross. Recent wolves: *a*, Greenland and Ellesmere land, $n = 12$ (ref. 20); *b*, Russia and north Scandinavia, $n = 4$; *c*, Denmark, Holocene and recent, $n = 7$ (ref. 20); *d*, Turkey, $n = 9$; *e*, Zagros mountains, $n = 5$ (ref. 6); *f*, Israel, $n = 26$; *g*, Arabian peninsula, $n = 7$. Fossil wolves and dogs: *h*, Ksar Akil U. Pleistocene, $n = 4$ (ref. 21); Tabun B, $n = 1$ (ref. 7); and Ein Gev IV, $n = 1$ combined; *i*, Mallaha mandible M_1 ; *j*, Hayonim terrace M_1 ; *k*, Palegawra *Canis* (ref. 6); *l*, Jericho Pre-Pottery Neolithic to Early and Mid Bronze Ages, $n = 7$ (ref. 17); *m*, Cayonu, east Anatolia, 9,000 BP (ref. 3); *n*, Korucutepe, east Anatolia, Bronze Age, $n = 12$ (ref. 22); *o*, Arad, north Negev, Early Bronze Age; *p*, Persian period of Israel, $n = 6$; *q*, two male dogs from Suez, Egypt; *r*, dogs from archaeological sites in Hungary, $n = 42$ (ref. 23). Recent jackals: *s*, Israel, $n = 24$.

The terrace of Hayonim, in the western Galilee, was excavated by O. Bar Yosef in 1966 and 1969, and by D. Henry in 1974–75 (ref. 14). Over a thin layer assigned typologically to the Geometric Kebaran A, lies a Natufian assemblage. From the lowermost level of this a single ¹⁴C date of 11,920 BP (SMU 231) is available. The canid tooth reported here is a lower carnassial from the trial trench (square D depth 30–50 cm, 1974 season's excavation) and is located within the Natufian. Its crown is chipped at the anterior end, and like the M_1 of the Mallaha mandible described above, is morphologically wolf-dog, with a poorly developed metaconid (Fig. 3).

The sizes of the M_1 's of both these finds are compared with other wolves and dogs both geographically with recent wolves from the Palaeartic region, and temporally with Near Eastern Upper Pleistocene to recent wolves and dogs (Fig. 4 and Table 1). The results of this analysis indicate firstly that recent wolves increase in size with increasing latitude in accordance with Bergmann's rule, and secondly that Upper Pleistocene wolves from the Levant were larger than recent ones, so that the wolf, like the mole rat, gazelle, goat, cat, fox, hyaena, wild boar and

Table 1 Recent and fossil canids, maximum length in millimetres of the lower carnassial, M_1

		N	Mean	s.d.
Recent wolves:				
Greenland and Ellesmere land ²⁰		12	29.6	1.46
Russia and north Scandinavia		4	29.7	1.67
Denmark, Holocene and recent ²⁰		7	29.3	0.76
Turkey		9	27.4	1.05
Zagros mountains, Iraq ⁶		5	26.6	1.7
Israel males and females		26	24.6	1.07
Israel males		25	25.0	1.54
Israel females		13	24.3	0.94
Arabian peninsula		7	23.2	0.73
Fossil wolves and dogs:				
Ksar 'Akil ²¹	Upper Pleistocene	4	28.8	2.66
Tabun B ⁷	Mousterian 40,000–45,000 BP	1	29.5	
Ein Gev IV	Geometric Kebaran 13,000–14,000 BP	1	28.6	
Ein Mallaha	Early Natufian c. 12,000 BP	1	22.6	
Hayonim terrace	Early Natufian	1	~22.2	
Palegawra ⁶	Zarzian 12,000 BP	1	21.9	
Jericho ¹⁷	Pre-Pottery-Neolithic-Bronze Age			
	10,000–4,000 BP	7	22.0	1.09
Cayonu East Anatolia ³	9,000 BP	2	(21.7 & 21.8)	
Korucutepe East Anatolia ²²	Bronze Age	12	21.7	1.53
Arad Israel, North Negev	Early Bronze Age 4,800 BP	1	20.3	
Israel	Persian period 2,500 BP	6	21.8	1.38
Male dogs, Suez, Egypt	Recent	2	(23.5 & 22.7)	
Archaeological sites in Hungary ²³	Neolithic–17th Century AD	42	20.7	1.62
Jackals, recent:				
Israel, males and females		24	18.7	0.88
Israel males		20	18.6	0.82
Israel females		12	18.6	0.89

auerochs decreased in size at about the end of the Pleistocene^{15–18}. Dogs from later archaeological sites in the Near East are even smaller than recent wolves. Thus three size classes of the wolf-dog line exist in Israel—Upper Pleistocene wolves, recent wolves and domestic dogs. The problem, therefore, is to which of the latter two size classes the Mallaha and Hayonim finds belong. Combining the two M_1 measurements, and comparing them with modern Israeli wolves, the probability that they belong to the latter is <0.01 (Student's t test) and we conclude that on this basis they are dog rather than small wolves.

Having distinguished three size classes for the Israeli Upper Pleistocene–Holocene wolf-dog line, we postulate two independent factors as having been responsible for each of the two size declines: (1) the temperature elevation at the end of the Pleistocene, which we associate with the difference between Upper Pleistocene and recent wolves, and (2) domestication resulting in the difference between recent wolves and dogs.

These finds are important in that they support evidence from other archaeological sites for interaction between man and canids in the Epipalaeolithic. The puppy, unique among Natufian burials, offers proof that an affectionate rather than gastronomic relationship existed between it and the buried person, an addition to our knowledge of the way of life of Natufian hunter-gatherers. The contemporary diminutive adult carnassials, of size equivalent to dog and not wolf, indicate that *Canis lupus* had, by the early Natufian, been subject to some degree of selective breeding by man. It would seem unlikely that taming alone would be sufficient to bring about this kind of change in the animals' dentition. We suggest, therefore, that domestication of the wolf occurred 12,000 years ago, before that of the food animals such as goat, sheep, cattle and pig, which were probably not domesticated until one or more millennia later¹⁹. If this hypothesis is correct, then together with Starr Carr in England, and Palegawra in Iraq, we might conclude that it was 'man-the-hunter' who first domesticated the wolf, another social carnivore.

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Note added in proof: The following dates pertaining to the puppy burial level at Mallah have been received from the University of Lyons radiocarbon laboratory: Ly 1660 11,590 $\pm$ 540 BP; Ly 1661 11,740 $\pm$ 570 BP; Ly 1662 11,310 $\pm$ 880 BP.

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Tumorous head is a maternal-effect homoeotic mutant of *Drosophila melanogaster*

THE homoeotic mutants of *Drosophila* are of special interest in developmental biology because they cause the substitution of one body segment by another in the adult. The antennae of *tumorous head* (*tuh*) flies are replaced by mesothoracic leg tissue and part of the head by genital structures^{1,2} (Fig. 1). Homoeotic mutants are thought to be the result of mutations in regulatory genes³ which cause a suppression of the activity of one set of genes and the activation of an alternative set to bring about such a specific, well-defined transformation. These mutations somehow cause a group of cells at some stage in the life cycle to change their developmental pathway and differentiate during metamorphosis into different structures from those which they would normally form. We have analysed the *tumorous head* mutant and report here that it is probably the consequence of a gene acting at the time of determination.

Homoeotic genes act at one of several possible stages in development to bring about the observed phenotypic changes³. They could alter the initial determination of the cells, so that early in development, around blastoderm formation, when cells are becoming committed to follow specific pathways in development they become leg and genitalia instead of antenna and head. Many homoeotic genes need to be active throughout development^{4,5}, so that when determined cells divide the daughter cells also have the same developmental commitment.

Fig. 1 a, Third antennal segment transformed to leg; b, rostral-haut transformed to genitalia.

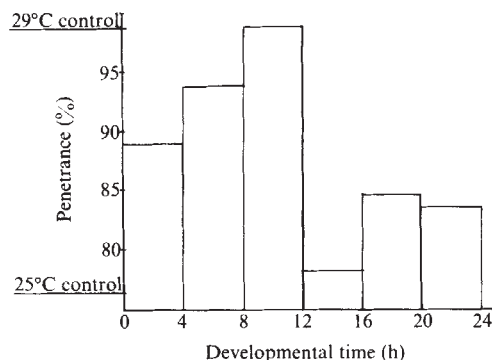
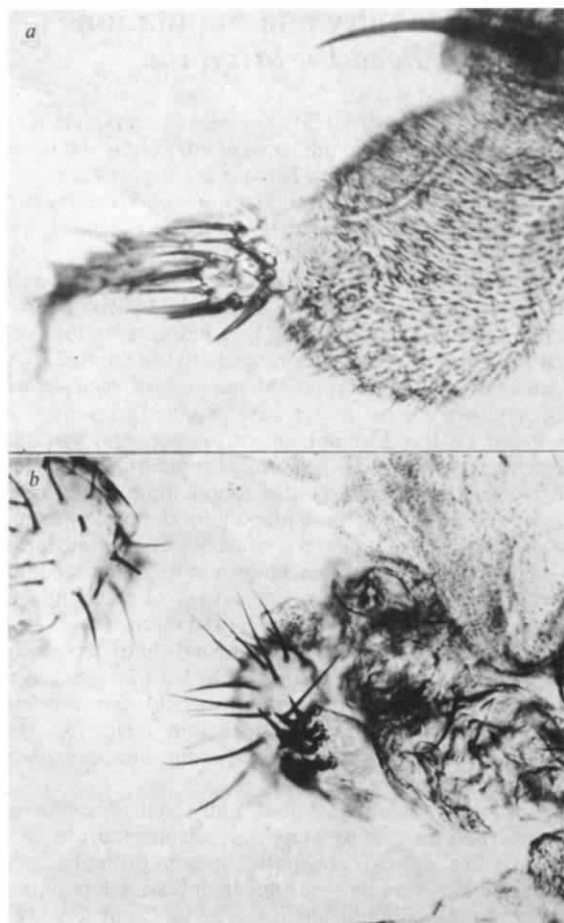


Fig. 2 Temperature sensitive period during embryogenesis of *tuh-3*. To establish a t.s.p. embryos laid by *tuh-3* mothers were shifted to 29°C for 4-h pulses. Embryogenesis in *Drosophila melanogaster* takes 24 h to complete, thus the following pulses were applied: 0-4, 4-8, 8-12, 12-16, 16-20 and 20-24 h after oviposition. Temperature shifts later in development indicated that there is no temperature sensitivity for post-embryonic stages. Between 100 and 400 adults were examined for each pulse applied and a similar response was obtained in 3 independent repeats of the experiment.

Thus, homoeotic genes could be envisaged which alter the maintenance of determination, in this case the cells would initially be determined correctly but because of the mutant gene products are unstable and change determination during development. Other genes could act at the time of differentiation by incorrect interpretation of the state of determination. *Bithorax* (*bx*) which transforms the haltere to wing is needed throughout development and probably affects the maintenance of determination^{4,5}. *Contrabithorax* (*Cbx*)⁶ which transforms the wing to haltere acts late in development and is also probably affecting the stability of cell determination.

The first few hours of embryogenesis in *Drosophila* are under the control of components laid in the egg by the mother⁷⁻⁹ and hence depend directly on maternal gene activity. Cells develop according to their location in the embryo^{10,11}. One could therefore expect that a gene affecting the initial determination of the cells would act during early embryogenesis or during oogenesis. A mutant in this class may well be a maternal effect mutation.

Table 1 *Tumorous head* is a maternal-effect homoeotic mutant

Female parent	Male parent	Number of flies scored	Penetrance (%)
<i>tuh-3/tuh-3</i>	wild type	323	18
wild type	<i>tuh-3/tuh-3</i>	465	0
<i>tuh-3/tuh-3</i>	<i>tuh-3/tuh-3</i>	714	53

Our experiments with *tumorous head* suggest that it is an unusual homoeotic mutation which fulfils the requirements of a mutant having its effect at the time of determination. There has been considerable genetic analysis of *tumorous head*, especially concerning the role of modifiers, enhancers and chromosome rearrangements^{12,13}. We took the original *tuh-1a*; *tuh-3* stock which contained some flies with tumorous head phenotype under the control of a third chromosome mutation (*tuh-3*) and a modifier on the first chromosome (*tuh-1a*). We isolated *tuh-3* from this stock and established a line free of known enhancers. Our homozygous *tuh-3* stock had a penetrance (percentage of flies showing tumorous head phenotype) of 70% in contrast to those of previous workers³ where the penetrance was 3%.

The *tuh-3* stock behaved as a maternal-effect homoeotic mutant. When homozygous *tuh-3* females were crossed to wild-type males some of the progeny showed the tumorous head phenotype but the reciprocal cross of *tuh-3* males to wild-type females gives no tumorous head phenotype. The gene also seemed to be active in early embryogenesis as *tuh-3* females

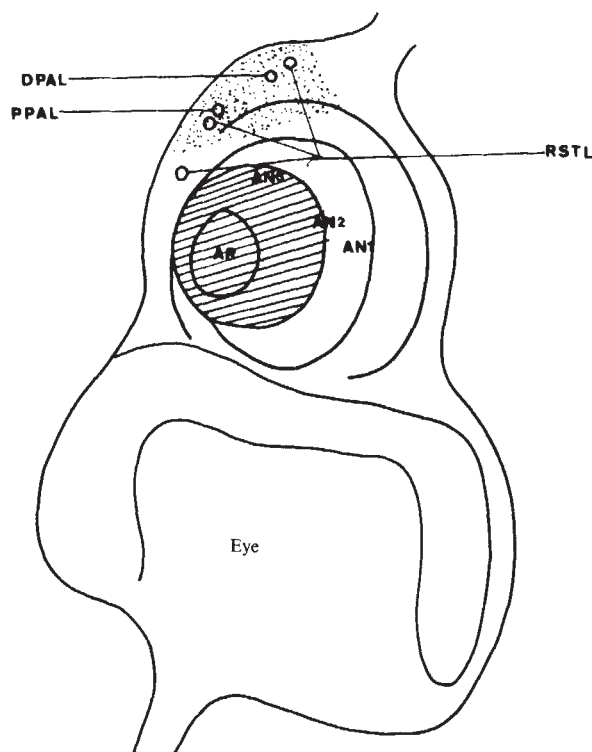


Fig. 3 Eye-antenna fate map indicating regions which give rise to the genitalia and leg transformations. Various regions of the disk were cut and implanted into host larvae where they metamorphosed with their host. Implants were scored for homoeotic transformations. In 33% of implants recovered from fragments containing the arista and third antennal segment we found leg tissue present and in 33% of implants recovered from fragments containing the rostralhaut region we found genitalia tissue present. RSTL, rostralhaut; AN1, AN2, AN3, 1st, 2nd and 3rd antennal segments respectively; DPAL, distal palpus bristles; AR, arista; PPAL, proximal palpus bristles. Stippled area, region that transforms to genitalia; hatched area, region that transforms to leg.

crossed to *tuh-3* males give more tumorous head progeny than when they are crossed to wild-type males (Table 1).

The temperature sensitive period (t.s.p.) of mutants is thought to be indicative of the time at which the gene products are being activated, thus the gene must be active before t.s.p. (refs 14,15). *Tumorous head* is temperature sensitive during embryogenesis and the pulse giving the highest *tumorous head* penetrance is 8–12 h after oviposition (Fig. 2). This then would agree with the *tuh-3*⁺ gene being involved in determination.

Interestingly *tumorous head* transforms cells in the eye-antenna imaginal disk into cells from two different disks located in different body segments¹⁶, the second leg disk in the mesothoracic segment and the genital disk in the last abdominal segment. All other known viable homoeotics act by altering cells from one disk to one other segment only^{15,17} (for example, *bithorax* transforms metathoracic disk cells into mesothoracic disk cells⁴) or to another compartment of the same structure (for example, *engrailed* transforms posterior wing cells into anterior wing cells¹⁸). The mutant *proboscipedia* transforms the proboscis to antenna or leg, but not simultaneously to both structures in one fly; and two lethal mutants have been described which transform from genitalia to leg and antenna¹⁹.

We have cut up the eye-antenna disks of *tumorous head* larvae (one can detect those with outgrowths in late third instar disks) and forced them through metamorphosis in a larval host²⁰. In this way we have mapped the area of the disk involved in the homoeotic transformation (Fig. 3). The two regions involved are in extremely close proximity in the eye-antenna disk, although not necessarily in the embryo²¹, but they undergo homoeotic transformations to very different segments of the fly.

As *tumorous head* is maternally controlled and has its t.s.p. early in embryogenesis, we would like to suggest that it is a candidate for a homoeotic gene acting at the level of embryonic determination.

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Genetic variability and population structure of *Poecilia latipinna*

THE pattern of genetic variability among natural populations is commonly attributed to the influence of environmental heterogeneity on natural selection^{1,2}. However, I report here that in *Poecilia latipinna* (Pisces: Poeciliidae), a small ovoviviparous fish, genetic variability is more closely related to local population structure, in particular, the adult sex ratio.

P. latipinna inhabits the brackish water lowlands along the Atlantic and Gulf coasts of the US and Mexico. Fourteen population samples were collected by hand seine in the northernmost two-thirds of the species range between South Carolina and Texas. For each population, the numbers of each sex were counted, and phenotypic males were assigned to one of four classes based on the development of primary and secondary sexual characters. These classes were: (1) immature male—anal fin only partially transformed into a gonopodium (male copulatory organ); (2) mature male—gonopodium completely formed, but no secondary sexual characters acquired; (3) transformed male—mature individuals possessing one or more secondary sexual characters: elongation of second ray of pelvic fins, and anterior displacement of pelvic girdle; (4) dominant male—fully developed complement of secondary sexual characters, including a large, sail-like dorsal fin (fin rays at least long enough to reach the base of the caudal fin). Both field and laboratory observations have shown that the sailfin character has a significant role as an indicator of social status during sexual and agonistic encounters^{3,4}.

The presumptive genotypes of all individuals in each population were determined by starch-gel electrophoresis of 21 cellular enzymes and two nonspecific muscle proteins. Seventeen allozyme loci were polymorphic in at least one population (frequency of most common allele was 0.99). On average, 43%

of the loci were polymorphic in each population. Within populations, the genetic variability (expressed as $\bar{H}$, the mean proportion of heterozygous loci per individual) was 3.6–10%, which is comparable to similar measures in other fishes⁵.

The extensive geographical range of *P. latipinna*, encompassing a diversity of temperate to tropical climates, offers an excellent opportunity to investigate the influence of environmental heterogeneity on genetic variability. However, the pattern of population-level genetic variability does not seem to be a simple function of any discernible environmental gradient. The regression of $\bar{H}$ against 13 climatic variables (water temperature—mean high, low and range; water density—mean high, low and range; rainfall; pan evaporation; heating degree days, 18 °C baseline; solar radiation; length of breeding season—water temperature >20 °C (ref. 6); number of days with temperature above 32 °C or below 0 °C)^{7,8}, both singly and in combination, did not show any statistically significant relationships. On the contrary, inspection of the geographical distribution of genetic variability (Fig. 1) shows that low levels of $\bar{H}$ can occur in both equable (KW) and rigorous (MB) extremes of the species range. Similarly, both highly seasonal (MB, MC, RV) and highly stable (KW) environments display reduced genetic variability. The San Marcos population (SM), which shows the highest level of genetic variability in the species ($\bar{H} = 10.0$), occupies a remarkably stable hot-springs habitat (annual temperature range 22–22.5 °C)^{9,10}. In light of such contradictory results, one might reasonably conclude that genetic variability in *P. latipinna* is responding to some unrecognised factor which is not an obvious component of local environmental heterogeneity.

The measure which successfully reflects interpopulation changes in $\bar{H}$ is the sex ratio of breeding individuals, which in this study is calculated as the number of females per dominant, or sailfin, male (Fig. 2; Kendall's rank correlation = -0.65, 0.001 < *P* < 0.01). In *P. latipinna* populations, the number of dominant males, both past and present, can be reliably determined because the sailfin character is a permanent morphological change. In other poeciliid species which form male dominance hierarchies, the accessory display signal possessed by

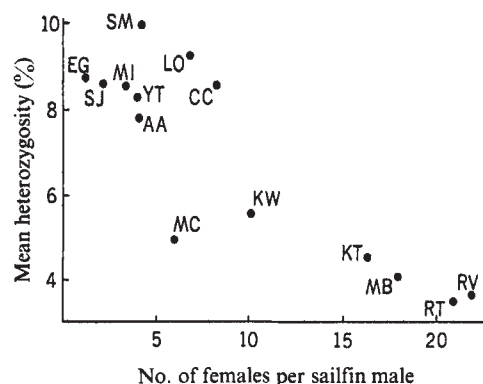


Fig. 2 Mean heterozygosity, $\bar{H}$, is inversely proportional to the ratio of females to dominant (sailfin) males.

high ranking males is usually some form of distinctive coloration^{11–19}, which may diminish, or even disappear, with the loss of social status^{13–19}. The number of dominant males in a population is of interest because many investigators have proposed that social dominance reinforces male reproductive success, even to the extent that dominant males may nearly monopolise the insemination of females^{13–19}. If this concept of dominance and its role in the regulation of mating activity is applied to *P. latipinna*, then the explanation for the relationship between sex ratio and $\bar{H}$ is straightforward: as the proportion of dominant males in a population decreases, the paternal genetic contribution becomes channelled through fewer individuals. Thus, for any constant population size (*N*), as the sex ratio of breeding individuals becomes more skewed, the local effective population size (*N_e*) declines²⁰, and consequently genetic variability is reduced^{21–23}. Previously, population size reduction^{24,25} and reproductive behaviour^{26–28} have been used to explain low levels of genetic variability in other organisms, but in *P. latipinna* the ratio of females to dominant males combines both of these concepts in a single direct measure of population structure.

It is possible that the observed sex ratios are not reflections of the true population structure, but are merely the artefacts of sampling technique or differential habitat selection by the sexes (or social classes). In this study such effects are minimal because the shallow streams and ponds where *P. latipinna* is found are usually small enough to obtain thorough samples. Even if one accepts the observed sex ratio as an accurate representation of local population structure, it could still be argued that the range of genetic variability in these samples is primarily determined by differences in gross population size. Without a careful census or identification of reproductive units this proposition is difficult to contest. However, rough estimates of gross population size at each sample locality can be obtained by taking the product of population density and habitat area. Such calculations show that to a first approximation the expectations for the effect of gross population size on $\bar{H}$ are not fulfilled: the two largest populations sampled in this study (KW and RV) fall at the low end of the range of heterozygosity; likewise, the two smallest populations (EG and SJ) are among the most genetically diverse. Reproductive substructuring within large, continuous populations could account for some instances of low genetic variability^{29,30}, but it is improbable that these objectively smaller populations (EG and SJ) are part of a larger (but undetected) panmictic unit. Another alternative would be that $\bar{H}$ is a function of the number of individuals in each sample, but no correlation was found between these two factors (*r* = 0.19; *P* > 0.50).

The appearance of a close association between population structure and genetic variability suggests that the interactions which regulate the local abundance of dominant males may be important adaptive features of poeciliid population biology. However, sources of variation in population structure need not

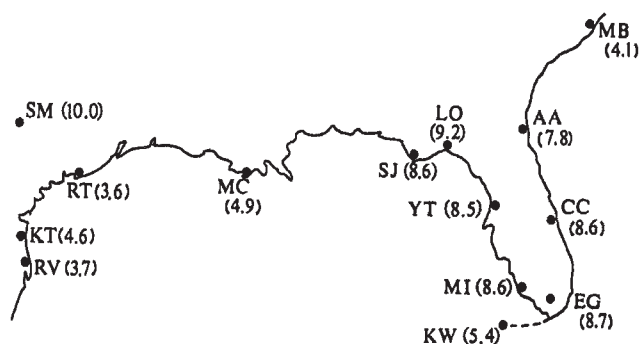


Fig. 1 The geographical location and the value of mean heterozygosity, $\bar{H}$, for 14 populations of *P. latipinna* from the Atlantic and Gulf coasts. Collection sites and sample sizes are as follows: South Carolina: Myrtle Beach (MB, 46); Florida: Jacksonville (AA, 53), Cape Canaveral (CC, 63), Key West (KW, 96), Everglades (EG, 25), Marco Island (MI, 50), Yankeetown (YT, 64), Live Oak Island (LO, 81), Port St Joe (SJ, 16); Louisiana: Morgan City (MC, 36); Texas: San Marcos (SM, 128), Rockport (RT, 61), Kingsville (KT, 73), Brownsville (RV, 256). Electrophoresis and enzyme assays performed on crude tissue extracts produced the following enzymes: eye esterase, lactate dehydrogenase (LDH); liver esterase, LDH, isocitrate dehydrogenase (IDH), phospho-gluco-isomerase (PGI), indophenol oxidase (IPO), peptidase (glycyl-tyrosine); muscle esterase, LDH, malate dehydrogenase (MDH), IDH, PGI, phospho-glucomutase (PGM), α -glycerophosphate dehydrogenase, creatine kinase, aldolase, peptidase (glycylglycine), nonspecific muscle protein. See ref. 4 for details of techniques.

be limited to intraspecific mechanisms. For example, studies of other poeciliids have shown that predatory fishes can significantly alter male demography by selectively feeding on the larger, showier males^{11,16,31}. As no large, potential predators were found to be sympatric with *P. latipinna* in these collections, the observed sex ratios are probably not greatly affected by this type of interaction.

In *P. latipinna* mean heterozygosity varies not with the degree of environmental heterogeneity, but with the proportion of dominant males in a population. This relationship is most easily explained in terms of differences in local effective population size brought about by the hierarchical regulation of male mating activity.

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Arginine vasopressin maintains ethanol tolerance

THE neurohypophyseal hormone, [Arg⁸]vasopressin (AVP), like some of its analogues and smaller peptides of related structure, has the capacity to influence memory-related processes through direct action in the central nervous system^{1–4}. These peptides have relatively long-lasting effects on memory consolidation or on retrieval of information^{1,5}. It has been suggested that development of functional tolerance to drug effects involves an adaptive mechanism in the CNS similar to that which occurs during learning and/or memory consolidation^{6,7}. We therefore wished to extend our studies⁴ of the central actions of the neurohypophyseal hormones to include an investigation of the influence of these peptides on various aspects of functional tolerance to ethanol. One of the most consistently demonstrable effects of the neurohypophyseal peptides with respect to memory is the ability to inhibit the extinction of a conditioned avoidance response^{1–3,5}. We now present evidence that treatment of animals with AVP during and after chronic ethanol

administration leads to long-term maintenance of tolerance to the hypothermic and sedative effects of ethanol.

Male C57BL/6 mice were fed a liquid diet containing 7% (v/v) ethanol or an equicaloric amount of sucrose (controls) for 7 days⁸. On the morning of the eighth day, all mice were offered the sucrose-containing liquid diet (withdrawal). Using this regimen, we have previously shown that tolerance to the hypothermic and sedative effects of ethanol and signs of physical dependence were evident after withdrawal of ethanol from the animals' diet⁸. In the present experiments, animals received daily subcutaneous (s.c.) injections of 10 µg of AVP or oxytocin in 0.1 ml saline, or saline alone, throughout the drinking period and for 9 days after withdrawal. Animals were not injected with peptides on the day on which the ethanol-containing diets were removed, since the animals undergoing withdrawal were subject to handling-induced seizures. Four groups of animals were included in each experiment. The first group consumed the sucrose-containing diet and received saline injections. The second group consumed the sucrose-containing diet and received hormone injections. The animals consuming the ethanol-containing diet were similarly divided into a group receiving saline injections and a group receiving hormone injections. During the drinking period, ethanol consumption, intoxication and blood alcohol levels^{8,9} were monitored daily. After removal of the ethanol-containing diet, withdrawal hypothermia and behavioural hyperexcitability⁸ were observed at 2-h intervals for 12 h and blood alcohol levels were determined at 2-h intervals for 6 h in certain animals⁹. Tolerance to ethanol, determined by the hypothermic response and duration of loss of righting reflex after an intraperitoneal (i.p.) injection of 3 g per kg ethanol⁷ was evaluated 24 h after withdrawal, and at 3-day intervals (starting on the third day after withdrawal) for 18 days thereafter. During tolerance testing ethanol metabolism was monitored in randomly selected mice from each group.

Blood alcohol levels during the drinking period and the clearance of alcohol after withdrawal and during tolerance testing were similar in all animals receiving the ethanol-containing diet. Signs of physical dependence, evident in the mice after ethanol withdrawal, also seemed to be similar in animals given saline, AVP or oxytocin¹⁰.

Functional tolerance was first determined at a time when overt withdrawal signs had subsided (24 h after withdrawal). Figure 1 shows the time course for disappearance of tolerance after animals were withdrawn from the ethanol-containing diet. At 24 h and 3 days after withdrawal, the response to the challenge dose (3 g per kg) of ethanol was significantly less, both in terms of hypothermia (Fig. 1a) and 'sleep time' (Fig. 1b), in all groups of animals which had consumed the ethanol-containing diets as compared to their respective controls. The response of control animals which had received AVP treatment was not significantly different from that of control animals which had received saline at any time tested (Fig. 1); the same was true for control animals which had received oxytocin (data not shown).

At 6 days after withdrawal of ethanol, animals injected with saline were no longer tolerant to the sedative or hypothermic actions of ethanol (Fig. 1 and see ref. 8); that is, their responses were not significantly different from controls. Ethanol-consuming animals which had been treated with oxytocin also were no longer tolerant. In contrast, at 6 days after ethanol withdrawal, animals which had received AVP remained significantly tolerant to the effects of ethanol, as measured by both change in body temperature and sleep time in response to the challenge ethanol dose. This tolerance persisted during the time that the animals were treated with AVP (Fig. 1), and when AVP treatment was discontinued, at 9 days after withdrawal, ethanol tolerance disappeared with a time course similar to that seen in the saline and oxytocin treatment groups. Thus, at 3 days after the end of AVP treatment (12 days after ethanol withdrawal), animals still showed a significantly decreased response to the hypothermic and sedative effects of ethanol, but by 6 days after the end of AVP treatment (15 days after withdrawal), sleep time was not significantly different from that of controls, and the hypothermic

response was returning towards control values. At 18 days after withdrawal, no differences in response were seen between control and ethanol-treated animals injected with AVP (Fig. 1a, b).

Thus, while the half-life for disappearance of ethanol tolerance after chronic ethanol treatment was approximately 3 days in untreated⁸ or saline-treated mice, treatment with AVP maintained this tolerance for as long as hormone administration was continued. Since AVP treatment did not affect the rate of ethanol metabolism either during the drinking period, during withdrawal, or during tolerance testing¹⁰, it is apparently

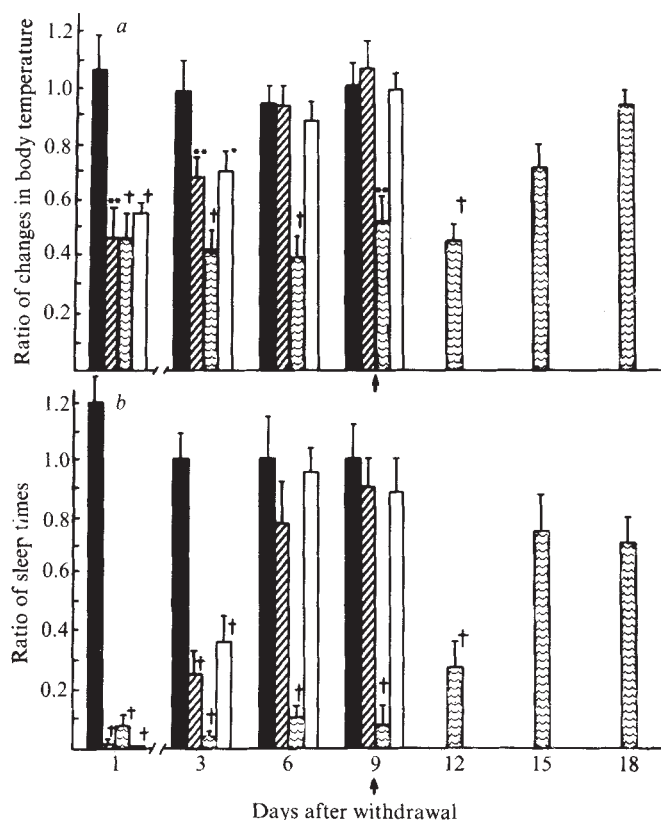


Fig. 1 Response of male C57BL/6 mice to a challenge dose of 3 g per kg ethanol, measured as decrease in body temperature (a) or duration of loss of righting reflex ('sleep time'; b). Data are presented as the ratio of the response of the ethanol-fed animals which had received various treatments to the response of their respective controls. The ratio of responses of control animals treated with AVP to those treated with saline is also shown. These ratios are less than 1.0 when the ethanol-withdrawn animals are tolerant to the effects of ethanol. As tolerance disappears the ratio approaches 1.0. Ratios were computed by dividing individual responses of animals in the group represented in the numerator by the mean response of animals in the group represented in the denominator; the value in the figure represents the mean $\pm$ s.e.m. of the ratios generated. ■, Controls: AVP-treated/saline treated. ▨, Ethanol-dependent, saline-treated/saline-treated controls. ▩, Ethanol-dependent AVP-treated/AVP-treated controls. □, Ethanol-dependent oxytocin-treated/oxytocin-treated controls. Significance was determined by comparison (*t* test) of each ratio with the ratio of AVP- to saline-treated controls. Ratios of ethanol-dependent AVP-treated to AVP-treated controls on days 12, 15 and 18 after withdrawal were compared to the AVP/saline control ratio on day 9 after withdrawal, the day that AVP administration was discontinued (†). †, $P < 0.001$; ††, $P < 0.01$; †††, $P < 0.05$. There were 6–12 animals in each group. The duration of the loss of righting reflex for saline-treated control animals when tested on day 1 after withdrawal was 25.1 ± 2.1 min and body temperature was depressed by $2.2 \pm 0.2^\circ\text{C}$ by the challenge dose of ethanol. These values for the AVP-treated control group were 25.8 ± 3.1 min and $2.1 \pm 0.2^\circ\text{C}$ and for the oxytocin-treated controls, 23.4 ± 2.8 min and $2.1 \pm 0.3^\circ\text{C}$. Similar values were obtained with animals of these groups at other times during the period of testing.

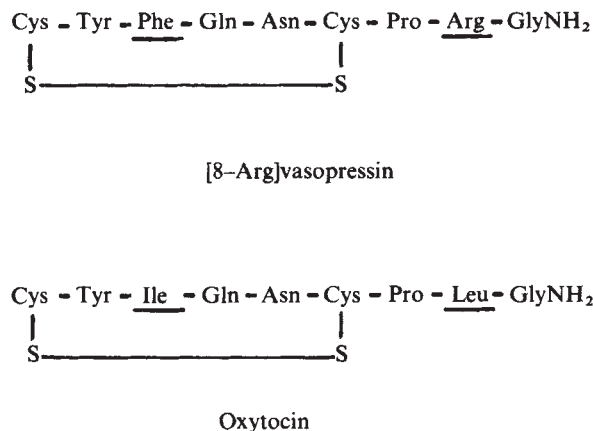


Fig. 2 Primary structure of arginine vasopressin and oxytocin. Nomenclature of peptides is in accordance with recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature²². AVP (430 IU mg⁻¹, rat pressor assay²³) and oxytocin (506 IU mg⁻¹, avian vaso-depressor assay²⁴) were supplied by Dr C. W. Smith (Department of Physiology and Biophysics, University of Illinois).

functional (CNS) tolerance to ethanol which is affected by the neurohypophyseal peptide. Treatment of the animals with an equimolar dose of oxytocin, a peptide which differs structurally from vasopressin by only two amino acids (Fig. 2), had no such effect on ethanol tolerance. These results are in agreement with studies on the effects of the peptides in inhibiting extinction of an active avoidance response¹, and with our previous studies regarding the ability of neurohypophyseal hormones to attenuate puromycin-induced amnesia in Swiss-Webster mice^{4,11}. In our studies, vasopressin attenuated the amnesia at a dose level at which oxytocin was ineffective¹¹. One has to consider that peripherally administered peptides which differ in their primary structure may be metabolised by different pathways¹² and may not be distributed in an equivalent manner within the CNS. On the other hand, oxytocin has been shown to modulate the development of functional tolerance to morphine in rats when administered s.c. at doses which were even lower than those used in our studies¹³.

The evidence that neurohypophyseal peptides affect memory-related functions by direct interaction with elements of the CNS has been previously reviewed¹. We have also found¹⁴ that after s.c. injection of a dose of ¹⁴C-labelled AVP¹⁵ comparable to that used in the present study, detectable amounts of radioactivity were present in brain as intact hormone; such results are consistent with an action of AVP in the CNS. Although the doses of AVP administered to our mice were high compared with circulating levels of this hormone, the rapid peripheral metabolism of the peptide hormones dictates the use of doses significantly higher than intraventricularly administered doses which are capable of producing similar behavioural effects. Direct interactions between ethanol and AVP could have contributed to the observed effect of the hormone on ethanol tolerance. During the drinking period, both AVP and ethanol were present in the animal simultaneously. However, further studies in our laboratory¹⁶ have indicated that when AVP injections are begun on the day after ethanol withdrawal, tolerance is still maintained for as long as the AVP treatment continues.

Acute exposure to ethanol inhibits the release of antidiuretic hormone¹⁷, and a recent study showed a rebound increase in urinary AVP excretion after clearance of a single dose of ethanol¹⁸. It seems that tolerance to the effect of ethanol on AVP secretion did not develop after chronic ethanol treatment¹⁸. Plasma AVP levels have been reported to be increased in rats at the end of a regimen of repeated ethanol treatments¹⁸ and in chronic alcoholic patients withdrawing from ethanol over a period of 5 days¹⁹. Furthermore, a selectively bred strain of alcohol-preferring rats, which was demonstrated to have both

higher innate tolerance and higher acquired tolerance to ethanol than outbred rats or a strain of alcohol-avoiding rats²⁰, has also been found to excrete more AVP during 6 h after ethanol intubation than the alcohol-avoiding rats²¹. Thus, the ability of AVP to maintain tolerance to the sedative and hypothermic effects of ethanol after chronic ethanol treatment, which was observed in the present study, may reflect a physiological role of the hormone in modulation of ethanol tolerance.

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Increased spontaneous activity following substance P infusion into A10 dopaminergic area

THE polypeptide, substance P, has a discrete regional distribution in mammalian CNS; particularly high concentrations are found in the mesencephalon, close to the origin of the two major ascending dopaminergic pathways^{1,2}. Two further observations which may reflect functional neurotransmission are, first, that substance P is released from spinal cord and hypothalamus^{3,4}, and second, that it has been localised using fragmentation techniques in nerve ending particles of bovine hypothalamus and substantia nigra^{5,6}. Further support for this hypothesis comes from iontophoretic studies in which application of substance P into the medial nucleus of amygdala and substantia nigra induced a strong neuronal activation^{7,8}. It has also been shown biochemically that the activity of dopaminergic systems may be modulated by substance P neurones^{9,10}. The ventral tegmental area (VTA), which contains the cell bodies of the dopaminergic A10 neurones (DA-A10), receives a substance P innervation mainly from substance P cell bodies localised in the

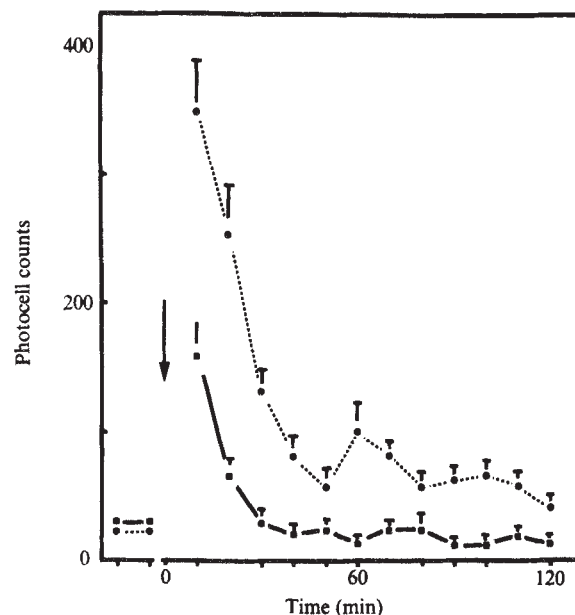


Fig. 1 Effect of acute bilateral infusion into the ventral tegmental area of isotonic saline (—) or substance P (---) on whole body locomotor activity measured in photocell cages. The ordinate indicates the mean photocell counts $\pm$ s.e.m., the abscissa indicates time by 10-min periods. Arrow, time of infusion. The lines before the arrow reflect baseline activity of the rats during 20 min before injection. The photocell cages were 70 cm $\times$ 25 cm and had two beams, which, when broken, registered counts in a recorder outside the testing room. Substance P was infused as a concentration of 3 μ g in 1 μ l ($n = 14$) and 1 μ l of saline was infused ($n = 12$).

medial habenular nucleus^{11,12}. DA-A10 neurones give rise to the mesolimbic DA system and also to the mesocortical DA axons innervating cerebral cortical area^{13,14}. We have studied the behavioural effects of local application of substance P into the VTA, to try to elucidate the physiological role of substance P innervation of this area. A strong enhancement of spontaneous activity was observed following bilateral infusion of substance P. A major feature of this enhancement was increased exploratory behaviour. Moreover *d*-amphetamine-induced behavioural arousal was potentiated by previous infusion (1 h) of substance P into the VTA.

Forty-three Sprague-Dawley rats (300-350 g) were implanted with bilateral stainless steel guide cannulae (23 gauge) aimed 3 mm above the VTA. Coordinates were (in mm): 3.5 posterior to bregma, 0.5 lateral from midline, and 5.6 below skull surface (incisor bar 5 mm above interaural line). Histological examination revealed that the 86 infusion sites were well localised in the VTA. Ten days after surgery, 3 μ g synthetic substance P or the solvent (1 μ l of 0.9% sterile saline) were injected bilaterally by lowering an injection needle (30 gauge) through the guide cannulae to a point 8.6 mm below skull surface; the infusion lasted 120 s and a 60-s diffusion time was allowed before removal of the needles. The substance P dose used in our experiments is considerably higher than the endogenous amounts of this substance in the VTA; however Jessel *et al.* have shown that there is no reuptake mechanism at substance P terminals and that substance P is rapidly catabolised by peptidases in the extracellular space⁴. The experiments were carried out during the least active part of the rat's circadian rhythm (1200-1700), with white noise (67-68 db), constant temperature (20 $^{\circ}$ C) and illumination (0.14 ft lamberts). Rats were familiarised to the experimental conditions before testing began.

In the first experiment, photocell cages were used to measure whole body locomotor activity. On a test day, the rats were habituated to the cages for 1 h; then saline or substance P infusion into the VTA was made and the rats were returned

immediately to the photocell cages for 2 h. Fourteen rats received synthetic substance P (Bachem) and 12 received saline. Animals injected with substance P in VTA exhibited a pronounced long-lasting increase in spontaneous activity (Fig. 1); analysis of variance indicated a significant group effect ($F = 28.4$, d.f. = 1,24, $P < 0.0001$) and group $\times$ time interaction ($F = 35$, d.f. = 11,11, $P < 0.001$). As soon as 20 s after the beginning of a substance P infusion, rats developed a behavioural excitation which was characterised by rearing, sniffing and hypermotility. The strongest effect was apparent during the first 40 min, but it was still in evidence during the subsequent hour.

In order to analyse this behavioural activation further, a second experiment was performed in a white square open field. Since rats have a tendency to avoid the centre area of the open field because of increased 'emotionality' or fear, we measured locomotion and rearing along the walls as well as in the centre area during a 10-min period. Eleven subjects were used as their own controls and were tested on four consecutive days. On the first, third and fourth test days, rats received bilateral infusion of saline into the VTA; substance P was injected on the second test day and all categories of behaviours analysed were enhanced after application (Fig. 2). Analysis of variance over 10 min revealed a significant activation of the following behaviours: locomotion periphery ($F = 5.4$, d.f. = 3,30, $P < 0.01$), rearing periphery ($F = 5.4$, d.f. = 3,30, $P < 0.01$), locomotion centre ($F = 13.9$, d.f. = 3,30, $P < 0.01$), and rearing centre ($F = 6.5$, d.f. = 3,30, $P < 0.01$). Interestingly, motor activity was not homogeneous throughout the open field, and that substance P infusion changed the spatial distribution of locomotion and rearing. The activity in the centre area in saline sessions was 7% of that in the periphery for both locomotion and rearing, whereas this was increased to 20% during the substance P session.

The examination of the drug $\times$ time interaction revealed a significant difference in the evolution of rearing between saline and SP sessions (rearing periphery $F = 2.4$, d.f. = 3,30, $P < 0.05$; rearing centre, $F = 4.4$, d.f. = 3,30, $P < 0.01$). This implies that although habituation was observed in saline sessions, during the substance P session rats remained activated throughout the 10 min. Individual comparisons between mean rearing scores on the last 5 min indicated a significant difference between substance P and all saline sessions (Newman-Keuls, $P < 0.01$). Twenty-four hours after the substance P injection motor behaviours in the open field were still elevated above control levels for the first 5 min of the session; but only the increased locomotion at the periphery was significant ($P < 0.01$).

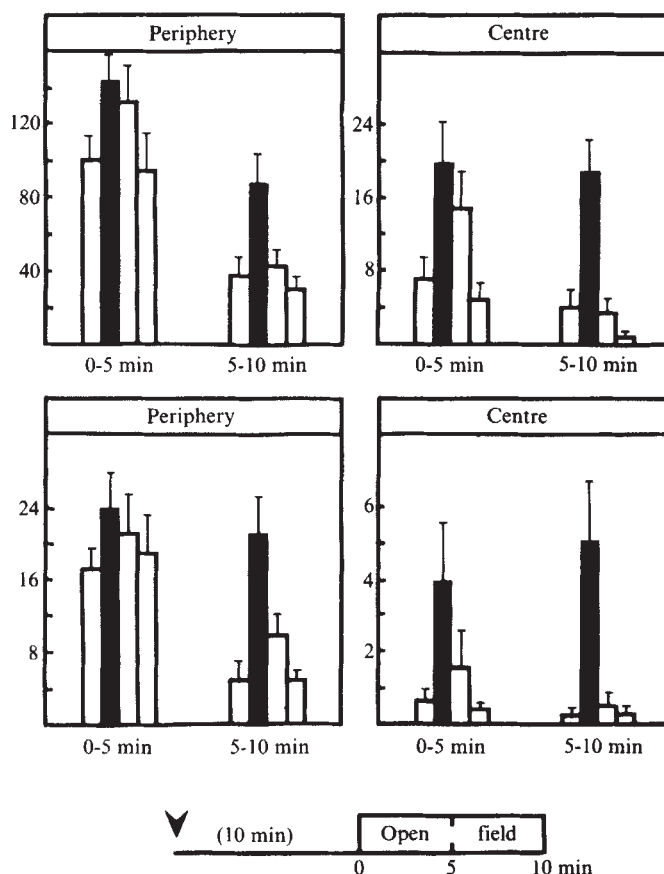
d-Amphetamine-induced locomotor activation is mediated by DA-A10 neurones¹⁵, and the magnitude of the response to *d*-amphetamine is determined by the firing rate of these neurones¹⁶. To evaluate a possible activation of DA-A10 neurones by substance P, we tested the reaction of 5 rats in photocell cages to a low dose of *d*-amphetamine (0.5 mg per kg i.p.) 1 h after bilateral saline (1 μ l) of substance P (3 μ g) infusion into the VTA. Four days elapsed between these two test sessions. Substance P significantly potentiated the effect of *d*-amphetamine (activity counts during 1 h after *d*-amphetamine: saline session 333 ± 123 , substance P session $1,503 \pm 473$, $P < 0.001$). The magnitude of this effect cannot be interpreted by the simple addition of the residual substance P effect and the *d*-amphetamine effect, since it is five times that observed after saline infusion.

One problem encountered with intracerebral infusions is that of diffusion to neighbouring structures; it is possible that the substantia nigra (SN), which contains a high amount of substance P, was affected by our injections. We have previously reported, however, that similar injections of substance P into the SN at a point 1.5 mm lateral to the VTA site elicit a syndrome of a quite different nature; consisting of stereotyped movements and, in particular, considerable self-grooming¹⁷. Infusion of substance P into the VTA induces a behavioural activation characterised by increased exploratory behaviour. An apparent

discrepancy to note is that one consequence of lesions of the DA-A10 neurones is a permanent hyperlocomotion¹⁸. This particular hyperlocomotion, however, is associated with decreased exploration and a disruption of goal-directed behaviour¹⁹. The activation induced by substance P is interpreted as an enhancement of purposeful and organised behaviours; lesion-induced hyperlocomotion could reflect an inability to attend or to 'focalise' behaviour.

It is possible that the potentiation of the *d*-amphetamine effect after infusion of substance P into the VTA results from increased firing rate of DA-A10 neurones. This is consistent with the ipsilateral increased release of newly synthesised ³H-DA observed in striatum by Cheramy *et al.*¹⁰, after application of substance P into the substantia nigra in the cat. Moreover, the *d*-amphetamine results indicate a long-lasting effect of substance P on DA-A10 neurones. Iontophoretic application of substance P in areas rich in substance P innervation shows a predominant excitation response with slow onset and long duration which is still present between 6 and 15 min after the end of the application⁷. Thus it is possible that VTA substance P-containing neurones, which originate principally in the medial habenular nucleus, exert a tonic activation on DA-A10 neurones. This hypothesis is strengthened by our recent results in which substance P-induced behavioural activation was

Fig. 2 Effects of acute bilateral infusion into the ventral tegmental area of isotonic saline or substance P on locomotion and rearing activities in the open field. Open bars indicate the saline test sessions (first, third and fourth days) and solid bars indicate the SP test session (second day). The locomotion (upper) histograms show the number of squares entered per 5-min period. The rearing (lower) histograms show the number of rears per 5-min period. The peripheral area was the band of squares adjacent to the walls; the centre area consisted of the inner squares. The arrow indicates that infusions were made 10 min before testing. The open field was 100 cm $\times$ 70 cm; the floor was divided into 20-cm squares and locomotion was measured by the number of squares entered. The central area was 60 cm $\times$ 60 cm. The rats were transported by hand and placed in the centre of the open field.



blocked by local application of haloperidol in the DA-A10 terminal areas, and by 6-hydroxydopamine-induced lesion of these terminals. Precisely which specific structures are involved in mediating the response to substance P cannot yet be determined. The medial habenular nucleus receives strong afferents from limbic structures such as septum, diagonal band of Broca and nucleus accumbens²⁰⁻²², all of which are innervated by DA-A10 neurones. The habenular nucleus also receives dopaminergic input from the VTA²³ and serotonergic afferents from the raphe nuclei²⁴ which are implicated in the control of DA activity^{25,26}. It is proposed that the substance P innervation of the VTA is part of a descending feedback loop which modulates the mesolimbic and mesocortical dopaminergic systems tonically.

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Denervation supersensitivity in the striatonigral GABA pathway

POSTSYNAPTIC receptors deprived of their associated presynaptic terminals exhibit denervation supersensitivity by showing enhanced responses to agonist drugs and elevated binding of radiolabelled ligands¹⁻⁴. To investigate whether this effect also occurs in γ -aminobutyric acid (GABA) neurones we have denervated the receptors for a well defined GABAergic projection in the rat brain, using the kainic acid lesion technique, and have found them to show enhanced responses to a GABA agonist drug and correlated elevations in radioligand binding.

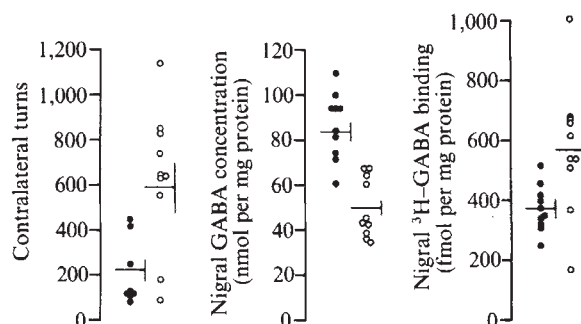


Fig. 1 Left: rotational responses in automated rotometers²⁰ 1 h after unilateral intranigral injection of muscimol (2 ng; stereotaxic coordinates: A2.2, V-2.8, L2.0, König & Klippel²⁹) made 3 weeks after ipsilateral striatal KA lesions (2.5 μ g; coordinates A8.0, V0.0, L2.5) or in unlesioned (control) rats. Intracerebral injections were made by slow-motion infusion in 1 μ l saline, under light ether anaesthesia for muscimol or barbiturate anaesthesia for KA, as previously described^{7,8,15}. Comparisons were by Mann-Whitney *U* test (2-tailed) ($P < 0.02$). Centre: nigral GABA concentrations in the substantia nigra of lesioned and unlesioned (control) hemispheres of rats 4 weeks after unilateral striatal KA lesions, determined by radioreceptor assay²². Comparison by Wilcoxon *T* test (2-tailed) ($P < 0.01$). Right: specific Na⁺-independent high-affinity ³H-GABA-receptor binding in Triton-treated membrane preparations of substantia nigra from lesioned and unlesioned (control) hemispheres of rats 4 weeks after unilateral striatal KA lesions, using ³H-GABA at 10 nM (ref. 21). Specific binding was defined as the difference between binding occurring in the presence and absence of an excess (100 μ M) of unlabelled GABA. Comparison by Wilcoxon *T* test (2-tailed) ($P < 0.03$). Protein contents of nigrae dissected from lesioned (116.5 $\pm$ 6.5 μ g) and unlesioned (113.5 $\pm$ 9.5 μ g) hemispheres were indistinguishable. ● Control; ○ lesion.

These results indicate that GABA receptors can show denervation supersensitivity and suggest that elevated GABA-receptor binding in Huntington's chorea may be of functional significance for the pharmacotherapy of this disorder.

Using the Ungerstedt rotating rat model⁵, in which the nigrostriatal dopamine system in one hemisphere is destroyed by intracerebral injection of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA), supersensitivity of denervated striatal dopamine receptors is manifested by a contralateral rotational response to peripheral administration of the dopamine-receptor agonist apomorphine. This has been shown to be associated with elevated binding of the dopamine-receptor ligands ³H-haloperidol³ and ³H-spiperone⁴, which correlates with the degree of apomorphine-induced turning⁴. The unilaterally 6-OHDA-lesioned rat has provided a useful animal model for the study of the physiology and pharmacology of dopaminergic mechanisms²⁻⁶. As GABA-dependent, dopamine-independent, contralateral rotational behaviour results from unilateral intranigral injection of GABAergic drugs⁷⁻¹², we sought to define a unilaterally GABA-lesioned model to facilitate the study of the physiology and pharmacology of GABAergic mechanisms in an analogous manner to the unilaterally 6-OHDA-lesioned rat. Thus, we destroyed the cell bodies of origin of the striatonigral GABA pathway^{13,14} and examined rotational responses to a GABA-mimetic drug and ³H-GABA receptor binding in the putatively denervated nigra.

Male Sprague-Dawley rats, 150-200 g, were given unilateral stereotaxic injections of kainic acid (KA, 2.5 μ g in 1 μ l saline) to destroy striatal perikarya, including the cell bodies of origin of the striatonigral GABA pathway^{16,17}. After 21 d rats were given unilateral intranigral injections of the potent and specific GABA agonist muscimol^{18,19} (2 ng 1 μ l⁻¹) ipsilateral to the KA lesion, and resulting rotational behaviour was quantified in automated rotometers²⁰. After a further 7 d rats were decapitated and nigral tissue dissected from each hemisphere from frozen sections. Specific ³H-GABA binding in Triton-treated membrane preparations of each nigra was determined using

^3H -GABA at 10 nM (ref. 21), and nigral GABA levels were determined using a sensitive radioreceptor assay²².

Unlesioned control rats showed contralateral rotation to this low dose of muscimol administered unilaterally into the substantia nigra⁷⁻¹¹. In successfully lesioned rats an enhanced rotation was seen when muscimol was administered into the denervated nigra (+173%, $P < 0.02$). In these rats, ^3H -GABA binding was greater in the nigra of the lesioned hemisphere than in that of the contralateral unlesioned hemisphere (+52%, $P < 0.03$), and GABA levels were reduced (-44%, $P < 0.01$) (Fig. 1). In lesioned animals there was a significant correlation between rotational response to intranigraly administered muscimol and elevation of nigral ^3H -GABA binding ($r = 0.686$, $P < 0.05$) (Fig. 2).

A Scatchard analysis of saturation data, carried out on the nigra of lesioned and unlesioned hemispheres, revealed that the elevation of ^3H -GABA binding in the nigra of the KA-lesioned hemisphere was primarily characterised by an 81% increase in maximal high-affinity ^3H -GABA binding (B_{max}), with no change in dissociation constant (K_D). B_{max} for low-affinity ^3H -GABA binding was elevated by 37% in the lesioned nigral preparations, with no change in K_D (Fig. 3). This profile of a denervation-induced increase in GABA-receptor sites, without a change in affinity for their ligand, is qualitatively similar to that seen in the dopamine-denervated striatum using ^3H -haloperidol as a dopamine-receptor ligand³.

Rats with denervated nigral GABA receptors showed an enhanced rotational response to the GABA agonist muscimol and elevated nigral binding of ^3H -GABA; rotational responses and elevation of ^3H -GABA binding were significantly correlated. These findings are consistent with the development of denervation supersensitivity of nigral GABA receptors deprived of their associated presynaptic terminals by destruction of their cell bodies of origin. This GABA-dependent rotational behaviour has been shown to be independent of nigro-striatal dopaminergic mechanisms⁷⁻¹², although these effects of GABA agonists may mimic those of dopamine agonists in the 6-OHDA-lesioned rat (that is, induce contralateral rotation) by acting beyond the striatum, at the level of the substantia nigra; the striatonigral pathway has been shown to constitute the striatal output system^{23,24}. We therefore propose that our study demonstrates a GABA-dependent rotational behaviour model in which GABAergic parameters, including correlated behavioural and receptor-binding indices of supersensitivity after unilateral lesions of descending GABA pathways, are similar in many respects to their equivalent dopaminergic parameters in the dopamine-dependent rotational model²⁻⁴.

Fig. 2 Correlation between rotational response 1 h after 2 ng muscimol, administered unilaterally into the substantia nigra and elevation of nigral ^3H -GABA-receptor binding, after ipsilateral striatal KA lesions. Significant correlation: $r = 0.686$, $P < 0.05$ (2-tailed). Line of best fit indicates a value of rotation (≈ 400 turns h^{-1}) for zero change in binding that is within the control range of turning for this dose of muscimol administered unilaterally into the substantia nigra of unlesioned rats (see Fig. 1).

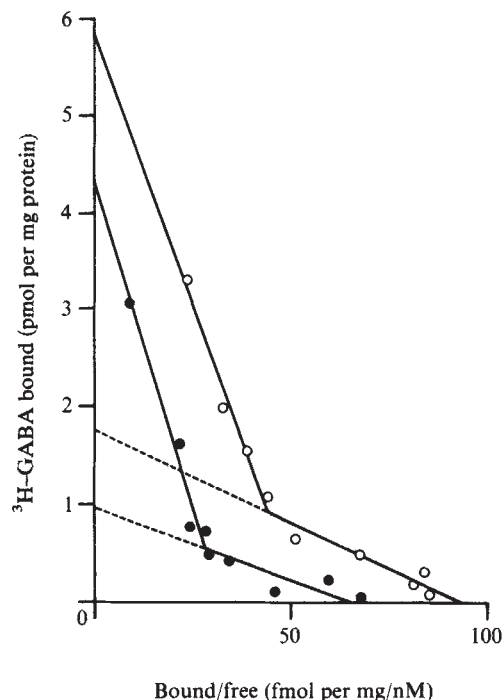
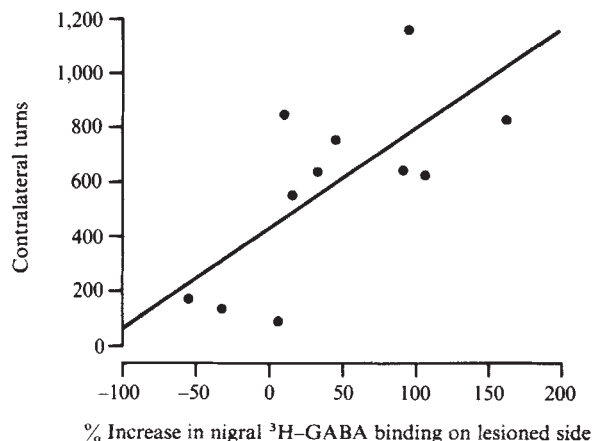


Fig. 3 Scatchard analysis of saturation data from ^3H -GABA-receptor binding carried out as described in Fig. 1 using 1–100 nM ^3H -GABA, on substantia nigra from lesioned and unlesioned (control) hemispheres of rats with unilateral striatal KA lesions. Maximum binding (B_{max} , fmol per mg protein) and dissociation constant (K_D , nM) for high-affinity ^3H -GABA binding were 958 and 15, respectively, in control animals (●), and 1,735 and 18, respectively, in lesioned animals (○). Respective B_{max} and K_D values for low-affinity ^3H -GABA binding were 4,234 and 130 in controls, and 5,831 and 112 in lesioned animals. Results are from pooled tissue of a further 6 lesioned animals.

The results demonstrate for the first time a correlation between receptor supersensitivity as indexed by a behavioural test and a ligand binding technique in a non-dopaminergic system. This model may be useful for studying the physiology and pharmacology of GABAergic mechanisms.

In addition, the striatal KA lesion has been proposed as a biochemical^{16,17} and behavioural²⁵ model for Huntington's chorea. Our demonstration of GABA supersensitivity in the substantia nigra after such lesions provides additional support for the validity of this model, as the substantia nigra in post-mortem brains from choreic patients shows elevated ^3H -GABA binding²⁶. Our study also suggests that, contrary to previous suggestions²⁷, GABA agonists may be of limited therapeutic potential in this condition. By acting on such supersensitive nigral GABA receptors at the level of the striatal output system, they may mimic those effects of increased dopaminergic transmission that are thought to be detrimental to clinical state in Huntington's chorea²⁸.

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Calcium-dependent exocytosis in bovine adrenal medullary cells with leaky plasma membranes

SECRETION from nerve terminals and many other cell types occurs by exocytosis, a process in which the contents of small intracellular vesicles are released after fusion of the vesicle membrane with the plasma membrane of the cell. Exocytosis generally requires calcium ions^{1,2}, but little is known about the site at which these Ca ions act. The electrophysiological experiments of Katz and Miledi³ strongly point to an intracellular site, a conclusion that is supported both by the observation that transmitter release is increased after microinjection of Ca into the presynaptic terminal of the squid giant synapse⁴ and the finding that the Ca ionophore A23187 promotes secretion from a variety of cells⁵. Further progress has been

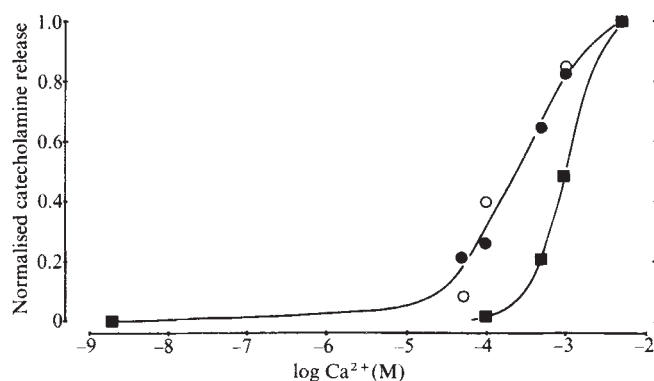


Fig. 1 Ca-dependence of catecholamine release from intact bovine adrenal medullary cells in response to 5×10^{-4} M carbamyl choline (●), 5×10^{-4} M veratridine (■) and to a potassium challenge (○). Ordinate: catecholamine release normalised to that in 5 mM Ca. Catecholamine was assayed fluorimetrically by the trihydroxyindole method⁷. Incubation was for 15 min at 37 °C after which time the cells were removed by centrifugation and samples of the supernatant taken for analysis. The medium contained (mM) NaCl, 136; KCl, 2.7; glucose, 5; Mg, 2; HEPES, 16 pH 7.2 and for the potassium challenge NaCl, 68; KCl, 70.7; glucose, 5; Mg, 2; HEPES, 16 pH 7.2. The media containing 2×10^{-5} M Ca²⁺ (calculated) contained in addition 5 mM EGTA.

severely hampered by lack of preparations in which this presumed intracellular site is freely accessible to experimental investigation. We now present evidence that the plasma membrane of medullary cells from the bovine adrenal gland can be rendered permeable to small molecular weight substances without blocking Ca-dependent exocytosis. This preparation has been used to examine the dependence of exocytosis on Ca and Mg ions.

Cells were obtained from thin slices of bovine adrenal medulla by protease digestion⁶. Catecholamine can be released in a Ca-dependent manner by stimulation with carbamylcholine, veratridine or potassium chloride. The Ca-dependence of catecholamine release in response to these secretagogues is shown in Fig. 1. In all cases secretion is half-maximal at Ca concentrations between 0.1 and 1 mM, and is associated with the release of the vesicular enzyme dopamine- β -hydroxylase but not cytosolic enzyme lactate dehydrogenase. Secretion is inhibited by the Ca channel blocker D600 (10^{-4} M).

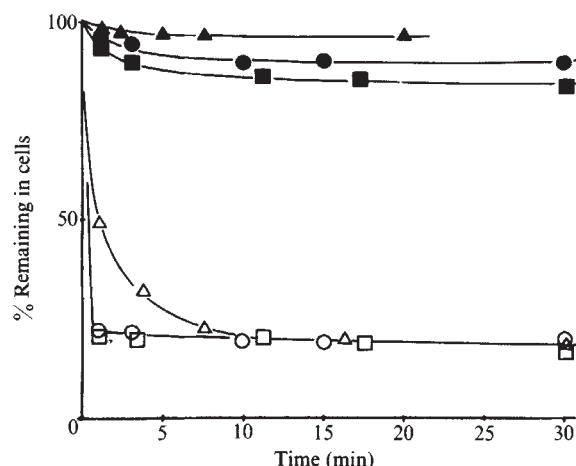


Fig. 2 Release of ^{86}Rb and catecholamine after exposure to high-voltage discharges. 1 ml of a suspension of medullary cells (10^6 per ml) preloaded with ^{86}Rb was placed between a pair of stainless steel electrodes (0.9 cm apart and each 1.0 cm^2) in a square perspex container and subjected to the discharge of a 2 μF capacitor. Cells exposed at zero time to 1 (▲, △), 5 (●, ○) and 10 (■, □) discharges, τ approx 200 μs . Field 2 kV cm^{-1} . Medium (mM): potassium glutamate, 138.7; glucose, 5; Mg, 2; PIPES, 20 pH 6.6; Ca-EGTA buffer, 5. Calculated free Ca^{2+} 10^{-5} M. Temperature 37 °C. Closed symbols represent catecholamine release and open symbols ^{86}Rb release. Similar results were obtained with cells preloaded with the non-metabolisable sugar 3-O- ^{14}C methyl-D-glucose showing that the increase in permeability is not restricted to charged molecules.

To provide ready access to the cell interior, suspensions of cells were exposed to a small number of high-voltage discharges⁸. This technique was used previously to alter the intracellular environment of erythrocytes^{9,10}. It presumably works by causing localised dielectric breakdown generating holes in the plasma membrane. This increase in permeability is most readily demonstrated in cells preloaded with ^{86}Rb where a suitable shocking regime can release 80% of the cellular ^{86}Rb within a few minutes (Fig. 2). As we wished to gain ready access to the cell interior for Ca-EGTA buffers, we also examined the uptake of ^{51}Cr -EDTA. In conditions similar to those used in Fig. 2, the ^{51}Cr -EDTA space increased within 5 min to a new steady level that varied between 50 and 75% of the cell volume. This suggests that the cells should have also been rendered freely permeable to Ca-EGTA which is only a little larger than Cr-EDTA. Although erythrocytes reseal after exposure to high voltage discharges, this does not seem to happen in adrenal cells where measurements of the ^{51}Cr -EDTA space show that the cells remain freely permeable for at least 20 min at 37 °C.

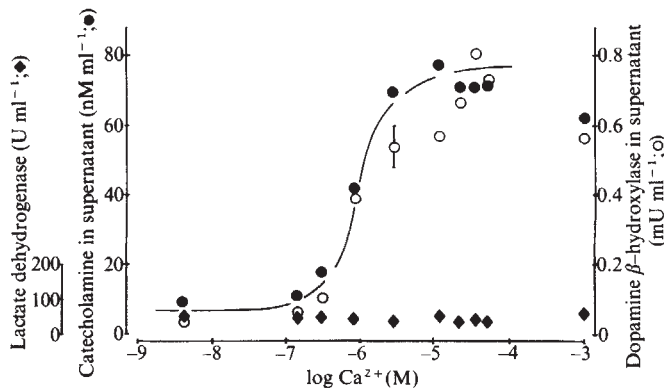


Fig. 3 Ca-dependence of catecholamine (●); dopamine- β -hydroxylase (○), and lactate dehydrogenase (◆) release in response to five discharges (2 kV cm^{-1} , $\tau \sim 200 \mu\text{s}$) over about 30 s. The samples were centrifuged and assayed 15 min after the discharges. Medium (mM): potassium glutamate, 138.7; glucose, 5; Mg^{2+} 2; PIPES, 30; Ca-EGTA buffer, 5; pH 6.6. Temperature 37°C . Catecholamine was assayed fluorimetrically⁷. Dopamine- β -hydroxylase was assayed spectrophotometrically by measuring conversion of tyramine to octopamine¹¹. Lactate dehydrogenase was assayed spectrophotometrically¹².

When cells are exposed to high-voltage discharges in media containing Ca, catecholamine is released (Fig. 2). This release is Ca-dependent, but is not mediated by depolarisation nor does it involve Ca channels. Release is not brought about by the temperature jump generated by the high-voltage discharge. Less than 1% of the total intracellular catecholamine is normally released after exposure to high-voltage discharges in nominally Ca-free media containing EGTA though the cells are rendered freely permeable to Cr-EDTA. With calcium present more than 10% of the total catecholamine is released and this response persists unchanged in cells fully depolarised by potassium and in the presence of a Ca channel blocker D600 (10^{-4} M). This Ca-dependent release of catecholamine seems to reflect exocytosis because it is associated with the selective release of the vesicular enzyme dopamine- β -hydroxylase but not lactate dehydrogenase which is present in the cytosol and is very temperature-dependent, being reduced by over 90% when the temperature is lowered from 37°C to 5°C . The catecholamine release is unlikely to result from a direct effect on the vesicles because exposure of isolated chromaffin granules to the same shock regime gives no release of catecholamine or dopamine- β -hydroxylase.

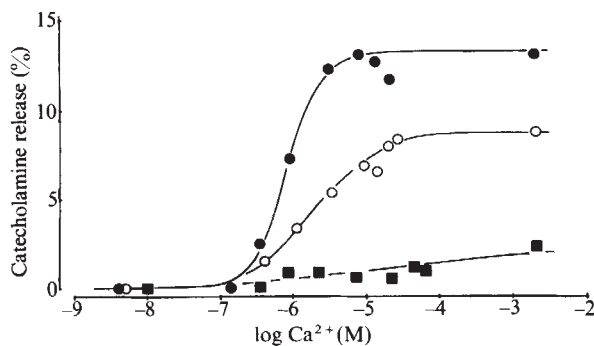


Fig. 4 Ca-dependence of catecholamine release at different magnesium levels in response to five discharges of 2 kV cm^{-1} . The samples were centrifuged and assayed 15 min after the discharges. Medium (mM): potassium glutamate, 138.7; glucose, 5; PIPES 20; Ca-EGTA buffer, 10.4, pH 6.6; MgCl_2 2, (●); 10, (○); 50, (■). Temperature 37°C . The time constant of the discharge is dependent on the conductivity of the medium and would have been close to $200 \mu\text{s}$ for media containing 2 mM MgCl_2 and $140 \mu\text{s}$ for media containing 50 mM MgCl_2 .

Working with cells rendered permeable by exposure to high-voltage discharges, we have investigated the dependence of exocytosis on the level of ionised Ca and Mg in the medium in which the shocks are delivered. Using two Ca-buffers, EGTA (Fig. 3) and EDTA, the Ca concentration giving half-maximal activation of the release of both catecholamine and dopamine- β -hydroxylase is close to $1 \mu\text{M}$ in the presence of 2 mM Mg . This is more than two orders of magnitude lower than the Ca concentration required for half-maximal activation of secretion from intact cells (Fig. 1), providing further evidence that exposure to high voltage discharges renders the interior of the cell freely accessible to externally applied Ca-buffers. Figure 4 shows that raising the Mg concentration both reduces the apparent affinity for Ca and also leads to a decrease in the total amount of catecholamine released.

Another interesting feature of Figs 3 and 4 is that only about 10% of the total cellular catecholamine and dopamine- β -hydroxylase is released even though the cells are freely permeable to Cr-EDTA. At first sight this is very surprising as one might expect that if Ca has free access to an intracellular site controlling exocytosis all the vesicular catecholamine and dopamine- β -hydroxylase should ultimately be released. There may, however, be only a limited number of release sites and the conditions used in our experiments may not permit repriming these sites for more than one round of exocytosis.

In all these experiments cells have been suspended in the test medium and then exposed to a series of high-voltage discharges.

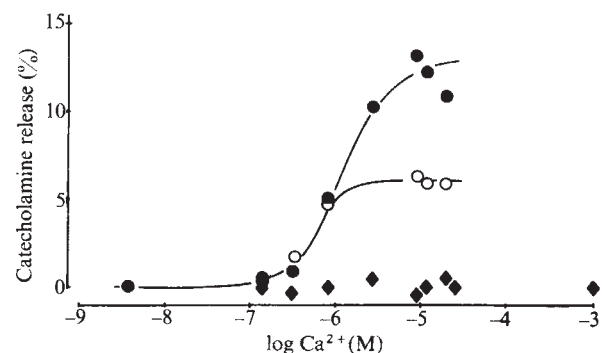


Fig. 5 Influence of ATP on Ca-dependent catecholamine release. Ten discharges 2 kV cm^{-1} , $\tau \sim 200 \mu\text{s}$ were applied to cells in a medium of (mM): potassium glutamate, 138.7; glucose, 5; PIPES, 20; EGTA, 0.4; ATP, 0 (●), 1 (○), 5 (■); pH 6.6. The cells were incubated for 30 min at 37°C and then Ca-EGTA buffer at pH 6.6 was added to give a final buffer concentration of 10.4 mM . After a further 15 min at 37°C the samples were centrifuged and assayed.

This means that the site at which Ca acts is still exposed to a fairly normal intracellular environment. In order to probe further the ionic and metabolic requirements for exocytosis it is necessary to render cells freely permeable in the absence of Ca and only expose them to Ca after adequate time has been allowed for small molecular weight materials to equilibrate. Figure 5 shows that this is technically possible. Preliminary results indicate that after exposure at 37°C for longer than 5 min to Ca-free solutions lacking ATP, addition of Ca fails to promote exocytosis whereas Ca remains fully effective for at least 30 min provided ATP is present throughout the period of exposure to nominally Ca-free media. These results suggest that ATP or something derived from it is necessary for Ca-dependent exocytosis in adrenal medulla cells. A similar conclusion had been reached from experiments on intact cells^{13,14}, but the present preparation seems to offer an excellent opportunity to discover the part played by ATP in exocytosis. Recently there have been a number of claims that Ca-dependent exocytosis can occur in cell-free systems¹⁵⁻¹⁷. A feature common to these systems and the present preparation is that all are activated by micromolar

concentrations of Ca which seems well within the physiologically possible range¹⁸. It is also within the range in which troponin and related Ca-binding proteins are activated.

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Depolarising effect of curare on embryonic rat muscles

CURARE (*d*-tubocurarine) reduces the responsiveness of skeletal muscle fibres to nerve stimulation¹ by acting as a competitive antagonist to the transmitter acetylcholine (ACh)². In adult muscle this action is not accompanied by any obvious change in muscle fibre resting potential, such as is produced by depolarising blocking agents³. We report here that in rat embryos curare evokes large depolarisations through action on ACh receptors.

Experiments were carried out on internal intercostal muscles from rat embryos (15–20 d gestation) and neonatal pups, with standard recording techniques⁴. During this developmental period intercostal muscles differentiate from primary myotubes to myofibres⁵. At all ages studied the muscle cells had resting membrane potentials of –70 to –90 mV in normal saline solution (see legend to Fig. 1), most displayed both spontaneous miniature endplate potentials (m.e.p.ps) and nerve-evoked endplate potentials (e.p.ps), and nerve stimulation produced contraction. When these preparations were perfused with medium containing 1–10 μ M curare (Calbiochem; Sigma), the recorded resting potentials fell to about –50 mV (Table 1), m.e.p.ps were reduced in amplitude or absent, evoked e.p.ps were small and the muscles gave only a weak contraction in response to nerve stimulation. When the curare was washed out the resting potentials recovered gradually and e.p.ps and m.e.p.ps reappeared. In some cases, we recorded from individual myotubes continuously during the application and washout of curare. The membrane potential in these fell from about –80 mV to about –50 mV in 10–14 min and returned to normal 16–20 min after washing. Presumably, most of this time was required for equilibration of curare in the bath.

Fibres depolarised by bath-applied curare did not desensitise. Reduced resting potentials ($\sim$ –50 mV) were still recorded after muscles had been in 10 μ M curare for 2 h or more. A similar absence of desensitisation has been reported for dissociated embryonic rat myotubes depolarised by ACh⁶.

A depolarising response was also seen when curare was applied focally from a micropipette. Glass microelectrodes were

broken to tip openings of 5–15 μ m and filled with a 0.5 mM solution of curare in saline; this was ejected by applying 0.5–1 kg cm^{–2} pressure in pulses of 0.1–2 s duration. Curare application by this means frequently produced localised regions of contraction in embryonic muscle. Intracellular recording revealed that during focal curare application the fibres are transiently depolarised by as much as 35 mV (Fig. 1). Some responses exceeded threshold and gave rise to action potentials (Fig. 1b). The amplitude of the response elicited was graded with the duration and pressure of the pulse and with the proximity of the curare pipette to the recorded myofibre. The effect did not seem to depend on the action of curare on presynaptic nerve terminals⁷, because (1) the depolarising response could be evoked by focal curare application anywhere along the length of the myotubes, (2) focal application did not increase the frequency of spontaneous m.e.p.ps before their abolition, and (3) the response was undiminished by the presence of 0.5 μ g ml^{–1} tetrodotoxin in the bath. As a control against possible artefacts arising from the pressure application technique, 15 fibres were subjected to similar pressure pulses from pipettes containing saline solution alone; none responded with depolarisations like those seen with curare (in several cases rapid depolarisations or hyperpolarisations of 2–5 mV were produced by the pressure pulse).

Two lines of evidence indicated that the effect of curare results from action on ACh receptors with a consequent increase in ionic conductance, as occurs during normal neuromuscular transmission. First, the effect of curare was abolished by preincubating the muscle in saline solution containing α -bungarotoxin, which binds ACh receptors with high specificity^{8,9}. Embryonic muscles (16 d) were incubated with 5 μ g ml^{–1} toxin for 2 h, the unbound toxin was washed out and myotube resting potentials were sampled before and after adding 10 μ M curare to the bath (Table 1). The curare had no effect on membrane potential after incubation in the toxin. Likewise, preincubation of muscles in the toxin prevented the phasic myofibre depolarisation and contraction evoked by focal application of curare (as in Fig. 1). Second, curare application reduced myofibre input resistance. Resistances were monitored by application of negative inward current pulses through the recording microelectrode, by means of a bridge circuit. After a single pulse of curare to the surface of myofibres, the input resistance dropped by up to 85% during the rising phase of the depolarisation (Fig. 1), and then gradually returned to its original value, 20–50 M Ω . In contrast, no comparable changes in resistance were detected when the fibres were depolarised (by as much as

Table 1 Membrane potentials recorded in various conditions from rat muscle fibres

Age	Membrane potential (mV)		
	Saline	Saline + curare	Saline + curare (preincubated in α -bungarotoxin)
15–16 d gestation	75.6 $\pm$ 0.9 (120/20)	48.3 $\pm$ 2.4 (21/3)	76.7 $\pm$ 1.7 (18/2)
17–19 d gestation	76.7 $\pm$ 0.6 (164/16)	47.0 $\pm$ 0.5 (130/15)	
0–3 d postpartum	70.1 $\pm$ 1.0 (52/8)	51.1 $\pm$ 0.8 (53/5)	
5 d–4 wk postpartum	63.6 $\pm$ 1.0 (18/2)	65.8 $\pm$ 0.6 (190/10)	
Innervated adult	84.3 $\pm$ 0.8 (35/3)		
Denervated adult	62.7 $\pm$ 0.9 (42/3)	64.8 $\pm$ 0.8 (56/3)	

The values indicate mean $\pm$ s.e.m. (no. of fibres per no. of muscles). Curare concentrations were 1–10 μ M when applied to embryonic and neonatal muscles, and 0.5 mM when applied to denervated muscles. Curare was applied 20–120 min before potentials were recorded. Muscles preincubated in α -bungarotoxin were exposed to 5 μ g ml^{–1} for 2 h.

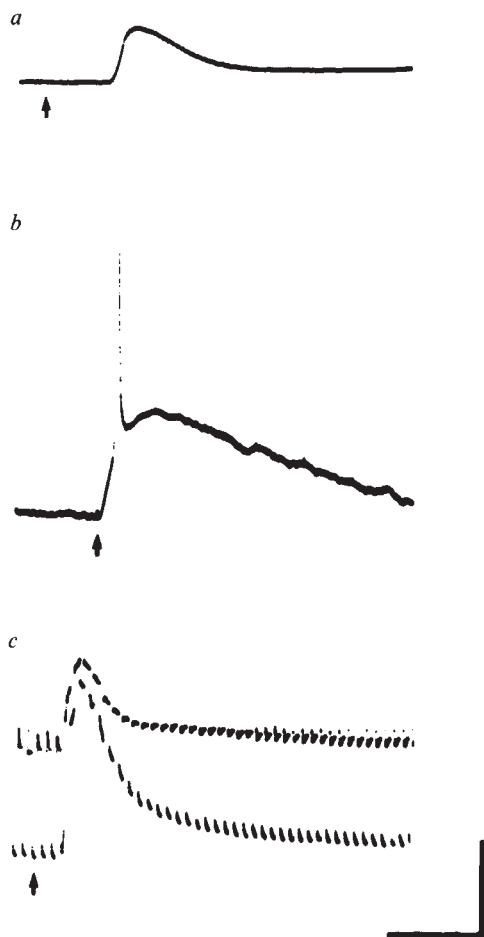


Fig. 1 Muscle fibre depolarisations evoked by focal curare application. Responses from different fibres, without (*a*, *c*) and with (*b*) an accompanying action potential. Curare was applied at the times indicated by arrows by the method described in the text. In *c*, fibre input resistance was monitored synchronously by injecting hyperpolarising current pulses of 50-ms duration and 1-nA amplitude at 300-ms intervals; the amplitude of the potential changes evoked by the current pulses was proportional to membrane resistance. It can be seen that the resistance decreased by 85% in this fibre during the curare response. Note that only partial repolarisations occurred during the intervals illustrated; characteristically, full recovery of the resting potential occurred within one or a few minutes of local curare application. Vertical calibration, 40 mV in *a* and *c*, 10 mV in *b*. Horizontal calibration, 4 s in *a* and *c*, 10 s in *b*. All experiments were done at room temperature (18–21 °C) in oxygenated saline containing 1–5% calf serum and (in mM): NaCl, 118; KCl, 3.0; CaCl₂, 4.0; MgCl₂, 1.1; glucose, 5.5; HEPES, 4.2 (pH 7.2).

50 mV) by raising external potassium to 50 mM through substitution for sodium. These results suggest that the curare acts on ACh receptors to produce an increase in membrane permeability.

The sensitivity of embryonic muscle fibres to curare disappeared at about the time of birth (21–22 d gestation). Of eight muscles examined 0–3 d postpartum, five were depolarised by 10 μ M curare in the bath and three were not. The phenomenon was not observed in 10 muscles examined $\geq$ 5 d after birth.

As neonatal rat ACh receptors have some properties in common with extrajunctional receptors of denervated adult muscle^{10–12}, we considered the possibility that adult extrajunctional receptors might also be depolarised by curare. We denervated intercostal muscles in three adult rats and one week later confirmed that they were supersensitive to focally applied carbamylcholine. However, none of the fibres sampled in these muscles was depolarised by 0.5 mM bath-applied curare (Table 1), and none of 10 fibres was depolarised by focally applied 5 mM curare. This agrees with previous studies of the blocking

action of curare on denervated rat diaphragm^{13,14}, in which no depolarising action was observed. By this criterion, then, the receptors of embryonic muscle differ from extrajunctional receptors of adult muscle.

Thus a depolarising response to curare seems to occur only in embryonic muscle fibres. One interpretation of this action is that a third type of ACh receptor exists on mammalian muscle, in addition to the well known junctional and extrajunctional receptors. Ritchie and Fambrough¹⁵ previously suggested that the “molecular identity of the receptors” on rat myotubes may differ from those on normal and denervated adult muscle, on the basis of reversal potentials for ACh-evoked responses. An alternative interpretation, which does not assume a new class of receptors, is that some feature of the membrane environment, such as fluidity, changes in a manner such that the same curare–receptor interaction initially has a channel-opening effect and later does not. Whatever the modification involved in the loss of responsiveness to curare, it must occur soon after birth. It is interesting that one week after birth there is also a marked shift in the gating properties of ACh-activated conductance channels in rat muscle¹⁶.

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Effect of tetraethylammonium on noradrenaline release from cat spleen treated with tetrodotoxin

EXPERIMENTS by Katz and Miledi¹ on the giant synapse of the squid stellate ganglion pretreated externally with tetrodotoxin (TTX) to block the conducted nerve impulses, and internally with tetraethylammonium (TEA) to block the potassium current, showed that a local regenerative response confined to the presynaptic terminal was obtained on depolarisation, provided that the external calcium concentration was sufficiently high. Strontium and barium substituted for calcium, and manganese and magnesium reduced the regenerative response. TEA, in the presence of TTX, also caused a massive release of acetylcholine from the frog neuromuscular junction in response to a depolarisation of the motoneurone terminals². These results offered direct evidence for the existence in the nerve terminals of a TTX-resistant calcium channel involved in transmitter release, and suggested that TEA, by allowing an inward calcium

Table 1 Effect of TEA on ^3H -NA release in TTX-treated spleen slices

	Fractional release per stimulus $\times 10^2$ (% of tissue ^3H -NA)				
	Krebs	TTX	TTX + TEA	TTX + TEA + lanthanum	
			(calcium free)		
^3H -NA	2.1 ± 0.4	0.3 ± 0.1	3.4 ± 0.7	0.2 ± 0.04	0.3 ± 0.1
	(12)	(12)	(9)	(9)	(9)

All solutions contained phenoxybenzamine ($1 \mu\text{g ml}^{-1}$). ^3H -NA was released by applying field stimulation pulses for 20 or 50 s at 1 Hz. Release per stimulus is expressed as a percentage ($\times 10^2$) of tissue ^3H -NA content at the time of stimulation. Concentrations: TTX, $0.32 \mu\text{M}$; TEA, 10 mM ; lanthanum, 1 mM . Numbers in parentheses refer to the number of experiments.

current to become regenerative, enhanced transmitter release. The release of noradrenaline (NA) from sympathetic nerves also requires calcium, and further, strontium and barium substitute for calcium in sustaining release, and magnesium, manganese and lanthanum block release³. Moreover, TEA has also been shown to enhance NA release after electrical stimulation of sympathetic nerves⁴⁻⁶. These observations suggest that a calcium channel also exists in the sympathetic nerve terminals and is responsible for the release of NA. The present investigation was undertaken to determine if in the presence of TEA and TTX, depolarisation of the sympathetic nerve terminal initiates a 'regenerative' inward calcium current which leads to an explosive release of NA. We report that it does.

Cat spleen slices (1 mm thick) were incubated in ^3H -NA solution (16 ng ml^{-1} , specific activity $10.43 \text{ Ci mmol}^{-1}$) to label the endogenous stores of NA⁷. A slice was then transferred to a superfusion chamber which consisted of two halves of plexiglass with a hollowed-out chamber of about 1 ml capacity. Two chloride-coated silver-wire coils, arranged in parallel, served as electrodes and the slice was wedged between them. The open ends of the chamber held small stainless-steel tubes to a polyethylene tube, through which the superfusion fluid was introduced at the bottom and collected from the top. Superfusion was started with oxygenated Krebs-bicarbonate solution containing phenoxybenzamine ($1 \mu\text{g ml}^{-1}$) at 35°C . The perfusion rate was about 8 ml min^{-1} . When spontaneous efflux of radioactivity had diminished to low and relatively constant levels, rectangular pulses (1 Hz , $20\text{--}80 \text{ V}$, 5 ms) were applied for 20 or 50 s. Effluent samples were collected for 2 min. Total tritium and ^3H -NA contents of the samples and the tissue were measured⁸. The increase in ^3H -NA release by nerve stimulation was obtained by subtracting the control background activity of the same volume of solution from the total output during stimulation. ^3H -NA release per stimulus is expressed as a percentage of the tissue radioactivity before stimulation.

Table 1 shows that ^3H -NA output due to field stimulation of sympathetic nerves was markedly reduced in the presence of TTX ($0.32 \mu\text{M}$). This blockade of release by TTX suggests that the field stimulation-induced release was mainly due to propagated action potentials. Residual release in the presence of TTX must be ascribed to the electrotonic depolarisation of the sympathetic nerve endings by current pulses applied by field stimulation, as in experiments in nerve terminals of the squid⁹. In the presence of TEA (10 mM), however, this residual response in the presence of TTX was restored to the control response obtained before TTX treatment. Secretory response in TEA plus TTX solutions occurred over a narrow range of voltages, and also at voltages much lower than those required to produce even a minimal response from preparations treated with TTX alone. This suggests that the release in the presence of TEA is not graded but may be triggered in a regenerative manner. Removal of calcium or addition of lanthanum (1 mM) abolished the response.

Our results show that in the presence of TEA, depolarisation of the sympathetic nerve terminals treated with TTX produced a massive release of ^3H -NA. The effect of TEA was restricted to

nerve terminals, as conduction of nerve impulses was never restored at a time when explosive release from nerve terminals by local depolarisations could be demonstrated. Katz and Miledi^{1,2} have previously discussed the possibility that inactivation of potassium efflux by TEA during depolarisation enables an inward calcium current to become regenerative. As TEA modifies NA release from sympathetic nerves in a manner comparable with its action on acetylcholine release from motoneurone terminals, it is possible that TEA may act similarly on the voltage-sensitive calcium channels in the sympathetic nerve terminals to facilitate calcium entry. We therefore suggest that one possible reason for the massive release of transmitter from TTX-treated preparations is the regenerative entry of calcium ions induced by the action of TEA on the sympathetic nerve terminals.

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Cell adhesion mediated by glycolipids

ADHESION between adjoining cells is a fundamental biological event, preceding both morphogenesis and organogenesis and also providing the basis for cellular recognition and membrane fusion¹⁻⁴. Adhesion is apparently mediated by carbohydrate moieties on the plasma membrane as shown in studies of sugar transferases⁵⁻⁷ and glycosidases⁸. A number of mechanisms such as the antigen-antibody reaction^{9,10}, the specific sugar transferase-substrate complex formation¹¹ and hydrogen bonding¹¹ between closely opposing carbohydrate chains have been proposed for the specific and nonspecific adhesion of cells, but the relevant surface molecules have not yet been isolated or identified. Two types of cell adhesion have been described: one is the reaggregation of dissociated animal cells in serum which may involve antibodies^{9,10} or unidentified components of serum such as the conglutinin¹². A second type of adhesion occurs in the absence of serum, and is probably regulated by glycoprotein factors^{13,14}. Recently we found that lipid particles containing phospholipids or glycolipids were agglutinated by serum and that glycolipid particles adhered most strongly to tissue culture cells even in the absence of serum¹⁵. We report here that lipids, in particular glycolipids, could mediate adhesion of animal cells.

To determine which particular glycolipids can mediate adhesion, a model system was used involving lipid particles and monolayers of tissue culture cells as adhesion partners. Glycolipids, either prepared by chemical synthesis or isolated from natural sources, were incorporated into lipid particles and tested for adhesiveness on various tissue culture cells (for experimental details see legends to Table 1 and Fig. 2). These experiments

showed that the lipid particles adhered most strongly to HeLa cells and less strongly to other cells in the following decreasing order: BHK > MDCK > 3T3 > MDBK > chick fibroblasts. These results suggest that the various cells contain differing numbers of combining sites in their plasma membranes. As the lipid particles were found to adhere most strongly to HeLa cells, these cells were used to test which lipids most effectively mediated adhesion. To measure the extent of adhesion, fluorescent lipids¹⁶, either dansylcerebroside or dansylsphingosine, were used. The relative adhesiveness of the different lipid particles,

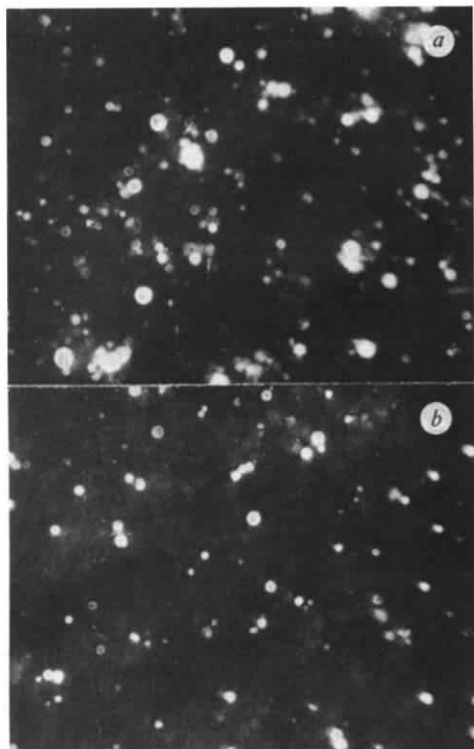


Fig. 1 Adhesion of glycolipid (ceramide-tetraoside, Table 1, compound L) particles to monolayers of HeLa cells and BHK cells. *a*, Adhesion to HeLa cells. *b*, Less extensive adhesion to BHK cells. To study adhesion, monolayers of tissue culture cells grown in Serva medium PM 13 to confluency in 5-cm diameter Petri dishes were switched to 2.5 ml of Dulbecco's medium³⁰ without serum. Aliquots of 0.1 ml of a suspension of glycolipid particles in PBS containing about 10^6 particles were added and the dishes were incubated for 2 h at 37° C. After the incubation period, the medium was decanted and the dishes were rinsed four times with PBS and observed using a UV microscope (Leitz).

measured by the intensity of their fluorescence, is summarised in Table 1. Comparable results were obtained when adhesion was estimated using a UV microscope. It can be concluded from Table 1, that although all lipid particles tested adhered to HeLa cells to varying extents, those particles containing galactose at the terminal positions were the most adhesive. Glycolipids with glucose, fucose, galactosamine or neuraminic acid at their terminal positions were less adhesive to monolayers. The galactose specificity is in accordance with the results of Chipowsky *et al.*⁷ and Weigel *et al.*¹⁸ who showed that 3T3 cells could most efficiently adhere to agarose beads which contained galactose as the ligand. Furthermore, the present experiments showed that adhesiveness increased with growing chain length of the sugar moiety. Glycolipids possessing four sugar molecules were the most adhesive of those tested—an exception was sulphatide, which is very adhesive although containing only one galactose molecule. The negative charge imparted by the sulphate group of the sulphatide does not seem to be the direct cause of its strong adhesion capacity, as other negatively charged lipids,

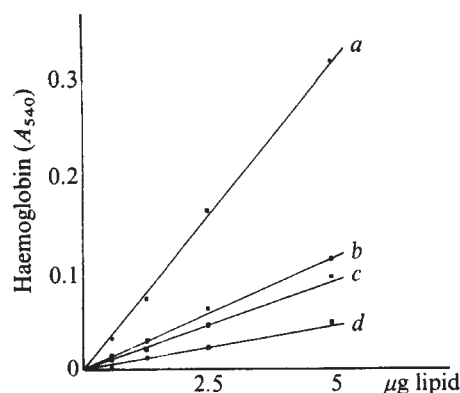


Fig. 2 Effect of glycolipids on the adhesiveness of erythrocytes to HeLa cells. *a*, Ceramide-tetraoside; *b*, oligoglucoside; *c*, sphingomyelin; *d*, phosphatidyl inositol. Most lipids listed in Table 1 could be dissolved or finely emulsified in PBS by ultrasonic treatment. In the case of ceramide monohexoside, solubilisation was aided by addition of Tween 20 at a detergent to lipid ratio of 1:1. 100 μ l of washed, neuraminidase-treated chick erythrocyte suspension containing 10 μ l of packed cells was incubated at 37° C for 45 min with 100 μ l of a PBS solution of lipid in the indicated amounts. After the incubation period, erythrocytes were washed three times with PBS. About 10^6 treated erythrocytes were incubated with HeLa cells as described for lipid particles (Fig. 1). The amount of adhered erythrocytes was determined as described in the legend to Table 1. Neuraminidase treatment of erythrocytes enhanced the effect of glycolipids. The standard condition of 0–5 μ g of lipid per 100 μ l of incubation mixture and an incubation period of 45 min was chosen, as a much higher concentration (>20 μ g per 100 μ l) of lipid and a prolonged incubation period (>1.5 h) may cause some haemolysis with long-chain glycolipid-treated erythrocytes.

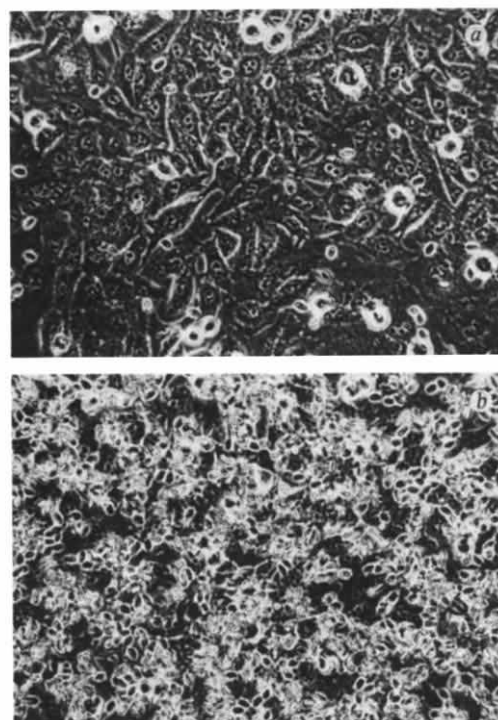


Fig. 3 Adhesion of erythrocytes to HeLa cells. *a*, Scant adhesion of neuraminidase treated erythrocytes to HeLa cells. *b*, Massive adhesion of neuraminidase treated erythrocytes, which have acquired a glycolipid (ceramide-tetraoside, L in Table 1) in their plasma membranes. Erythrocytes were incubated with 10 μ l of ceramide-tetraoside in 100 μ l of the incubation mixture for 45 min. Other methods as Fig. 2.

such as ganglioside or phosphatidyl inositol, did not adhere strongly. All the phospholipid particles tested adhered only moderately to HeLa cell monolayers. Nearly the same pattern of adhesion specificity with respect to the sugar moiety of glycolipids was obtained with BHK and MDCK cells. Adhesiveness of lipid particles to other cells was not rigorously quantitated.

To determine whether glycolipids could also promote adhesion between natural membranes, cells in which there was good correlation between adhesiveness and glycolipid content were required. Chick erythrocytes were satisfactory for this purpose, as they do not normally adhere to tissue culture cells but become artificially adhesive when glycolipids are incorporated into their membranes. The previously described

Table 1 Adhesiveness of synthetic and natural lipids to monolayers of HeLa cells

Lipids	Adhesiveness of	
	lipid particles (fluorescence intensity*)	erythrocytes (haemoglobin†)
A Ceramide-Glc	18	0.03
B Ceramide-Gal	25	0.04
C Ceramide-Gal-SO ₄ ⁻	91	0.25
D Ceramide-Fuc	20	0.01
E Ceramide-Glc-Gal	40	0.12
F Ceramide-Gal-Gal-Gal	71	0.23
G Ceramide-Gal-Gal-Gal-Gal	92	0.23
H Ceramide-Glc-Glc-Glc-Glc	49	0.12
I Ceramide-Glc-Gal-Gal-GalNAc	31	0.13
J Ceramide-Glc-Gal-Glc-Gal	90	0.27
K Ceramide-Glc-(NeuNAc)Gal -GalNAc-Gal-NeuNAc	37	0.18
L Ceramide-Glc-Gal-GalNAc-Gal	100	0.35
M Sphingomyelin	23	0.08
N Phosphatidyl cholin	20	0.07
O Phosphatidyl ethanolamine	21	0.07
P Phosphatidyl inositol	24	0.08

A, Isolated from a Gaucher spleen²³. B, Isolated from human brain²⁴. C, Isolated from human brain²⁴. D-H, Chemically synthesised by coupling ceramide with fucose, lactose, trigalactoside, tetragalactoside and tetraglucoside, respectively. Ceramide was obtained from brain cerebroside by the method of Carter *et al.*²⁵. L-Fucose and lactose were from Serva. Trigalactoside and tetragalactoside were obtained from gum arabic (Serva) by partial hydrolysis with 0.2 M HCl at 100°C for 2 h and isolation from a column of active charcoal²⁶. Tetraglucose was obtained from potato starch by the same procedure. Coupling of ceramide with oligosaccharides was performed via acetobromo derivatives of oligosaccharides with silver oxide as catalyst²⁷. By this procedure the oligosaccharides were exclusively linked to the C-1 of ceramide. The yields of glycolipids were in general 3–10% of the theoretical value. The products were purified by silicic acid chromatography using increasing concentrations of methanol in chloroform (10%, 25%, 50%), methanol and finally with 10% concentrated ammonia in methanol as eluting solvents. Fractions were monitored by thin layer chromatography using chloroform-methanol-water (65–35–8) as the developing solvent. I, Globoside, isolated from MDBK cell as the major glycolipid. J, Obtained by chemical coupling of ceramide with the corresponding tetrasaccharide isolated from human milk²⁸, using the same procedure as described for D-H. K, Ganglioside isolated from human brain²⁹. L, Ceramide-tetraoside. Obtained from ganglioside (GD_{1a}) by repeated hydrolysis with 0.02 M HCl at 80°C. The liberated N-acetylneuraminic acid and the HCl were removed by dialysis. Lipid particles containing these glycolipids or phospholipids were prepared essentially as described elsewhere¹⁵, except that ratio of the components of the particles were modified to glycolipid or phospholipid-cholesterol-triolein-dansylsphingosine 1:2:0.1:0.01. The technique of binding assay of lipid particles is described in the legend to Fig. 1. The extent of adhesiveness was measured by the fluorescence intensity of adhered lipid particles. To measure fluorescence, the washed monolayers were left with 3 ml of ethanol for 10 min at room temperature and the ethanol extracts were measured in a Perkin-Elmer fluorescence spectrophotometer. In the case of adhesion studies using erythrocytes, lipids were incorporated into erythrocyte membranes as described in Fig. 2 at 5 µg lipid per incubation mixture. The treated erythrocytes were then incubated with monolayers of HeLa cells as for Fig. 2. Adhesion was measured by the haemoglobin content of adhered erythrocytes. For this purpose, monolayers were scraped off the plates in 2 ml of phosphate-buffered saline (PBS). Haemoglobin was then liberated by repeated (3×) freezing and thawing. After centrifugation at 2,000g for 10 min the absorption of the clear supernatant was measured at 540 nm.

* Excitation at 340 nm and emission at 500 nm. All values were relative to that of compound L set at 100.

† Extinction at 540 nm.

method¹⁶ for the incorporation of glycolipid into natural membranes was used. As shown in Fig. 2 adhesiveness of erythrocytes was enhanced on incubation with increasing lipid concentrations. Although the mechanism by which lipid is incorporated in this system is not known, we conclude from these results that lipids, especially glycolipids can promote the adhesion of natural membranes. Figure 3 demonstrates the difference in adhesiveness of glycolipid treated and untreated erythrocytes on HeLa cells. In a subsequent experiment all the lipids listed in Table 1 were incorporated into erythrocytes in identical conditions in order to determine their relative efficacy in enhancing adhesion of erythrocytes to tissue culture cells. Adhesion was measured by following the haemoglobin content which remained associated with the HeLa cells after washing. Results presented in Table 1 (right vertical column) correlated favourably with those obtained with lipid particles (left vertical column); that is, glycolipids containing at least four sugar molecules and a terminal galactose stimulated the adhesiveness of erythrocytes most. Comparable results were obtained from two independent experiments, so the values in Table 1 seem to represent the real relative adhesiveness of different lipids. Because of these results it also seems unlikely that the values shown for the glycolipid-coated erythrocytes reflected differential equilibrium in the binding of glycolipids to erythrocytes.

Glycolipids are specific components of plasma membranes¹⁹. It is known that animal cells contain a mixture of glycolipids, some of which terminate with a galactose molecule²⁰. In the past, glycolipids have been considered to possess biological properties such as blood group specificity²⁰ or receptor functions^{21,22}, implying that they may be accessible to external factors. The present experiments have demonstrated a possible function of glycolipids in cell adhesion, an important property for the organisation of multicellular organisms.

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5,6-Dihydroxyindole is a melanin precursor showing potent cytotoxicity

WE have previously suggested that factors which regulate the proliferation and pigmentation of Cloudman S-91 mouse melanoma cells share common biochemical pathways^{1,2}. Our conclusion was based on the following observations: (1) most mutant cells selected for their ability to grow in culture medium containing melanocyte-stimulating hormone (MSH) show an altered ability to produce pigment¹, and (2) tyrosinase is activated in response to elevated levels of cyclic AMP in a reaction apparently mediated by a cyclic AMP-dependent protein kinase³. This same protein kinase has been implicated as a positive regulator in the proliferation of Cloudman cells⁴. In addition, we found that some precursors of melanin are cytotoxic and that cells actively producing melanin run the risk of self-destruction through a build-up of these precursors. We have previously shown that pigmented cells are killed as a result of exposure to excess tyrosine or dihydroxyphenylalanine (dopa) in the culture medium^{2,5}. It has since been demonstrated that actively melanising cells release a cytotoxic agent into their culture medium⁶. Here we present further evidence for the cytotoxicity of melanin precursors and show that 5,6-dihydroxyindole is one of the toxic compounds in the melanin biosynthetic pathway.

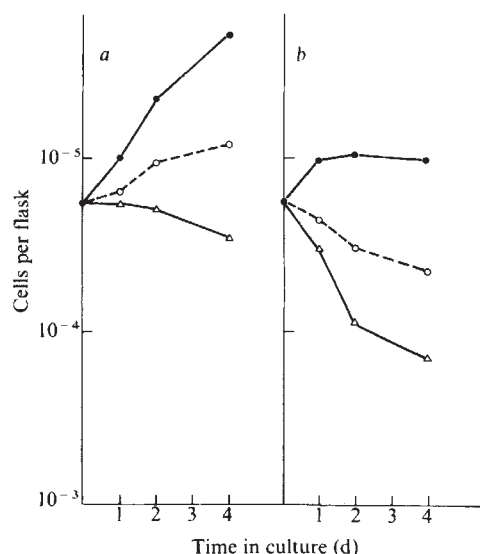


Fig. 1 Effects of dopa and MSH in combination with theophylline on cellular proliferation. Cells (2×10^4) were inoculated into Corning 25-cm² tissue culture flasks. The next day medium was removed and replaced with fresh medium containing no additions (●), 1×10^{-4} M dopa; (○), or 2.5×10^{-4} M dopa (△). *a*, Medium containing no MSH or theophylline. *b*, Medium containing MSH (2×10^{-7} M) and theophylline (5×10^{-4} M). At the times indicated cultures were collected in triplicate and counted in a Coulter counter. Variation within triplicates was less than $\pm 20\%$. These experiments were repeated three times with similar results.

From various clinical and experimental observations it was concluded that during melanisation, pigment cells produce substances which are potentially autotoxic, and it was predicted that a strong stimulus for the synthesis of melanin would raise the intracellular concentration of tyrosine, dopa and their derivatives and increase the likelihood of self-destruction of the melanocytes⁷⁻⁹. This prediction was supported by results of studies with cultured mouse and hamster melanoma cells. Tyrosine, dopa and tryptophan were toxic to melanotic cells but

Table 1 Effects of various amino acids on the viability of melanoma cells

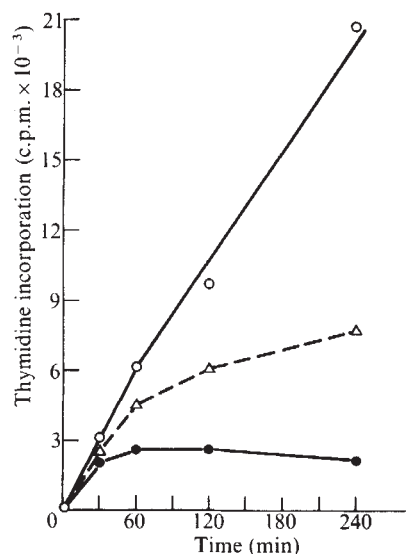
Additions to culture medium	Cell count (% of controls) after 6 d in culture	
	Melanoma cells	L cells
None	100	100
Ala, Arg, Asp, AspNH ₂ , Cys, Glu, GluNH ₂ , Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Val	100	100
Dopa	<1	65
Tryptophan	1	100
Tyrosine	12	100

Mouse L cells or Cloudman S91 melanoma cells (2×10^4) were inoculated into 25-cm² Falcon tissue culture flasks in 4 ml F10 culture medium. The next day the cells were fed media supplemented individually with each amino acid indicated. All concentrations of amino acids were 5×10^{-3} M except dopa (1×10^{-4} M). Media were changed every 2 d and on the 6th day the cells were collected and counted in a Coulter counter. Control cultures of L cells had a generation time of 22 h, and control cultures of melanoma cells had a generation time of 43 h. These experiments were repeated twice, with similar results each time.

not to amelanotic mouse L-cell fibroblasts, although dopa caused a slight retardation of the L-cell growth rate (Table 1). Other naturally occurring amino acids had little or no effect on either cell type. Cells with high tyrosinase activity that resulted from exposure to MSH were much more susceptible to the toxicity of melanin precursors than were cells not exposed to the hormone (Fig. 1 *a, b*). Treatment of cells with phenylthiourea, a potent and selective inhibitor of tyrosinase, provided complete protection from the toxicity of the melanin precursors². Together, these results indicate that one or more products of the tyrosine-tyrosinase reaction are cytotoxic and that for cultured Cloudman S-91 melanoma cells, the earlier predictions⁷ mentioned above are correct.

We next attempted to determine the nature of the compounds responsible for the cytotoxicity. Cultured cells were exposed for

Fig. 2 Effects of dopa and 5,6-dihydroxyindole on the rate of thymidine incorporation into acid-precipitable material. Cells were inoculated into Linbro wells as described in Table 2. At the times indicated the amount of ³H-thymidine incorporation into acid-precipitable material was determined in quadruplicate wells. Variation was less than $\pm 15\%$. The experiments were repeated twice, with similar results each time. ○, Controls; △, dopa (1×10^{-4} M); ●, 5,6-dihydroxyindole (1×10^{-4} M).



a few hours to various agents and their effects on the incorporation of ^3H -thymidine into acid-precipitable material were determined. In the first experiments cells were exposed to dopa ($4 \times 10^{-4}\text{ M}$) in the presence or absence of ascorbic acid ($1 \times 10^{-3}\text{ M}$) as a reducing agent¹⁰. After 16 h, cells in medium containing dopa alone were highly melanised and many were lysed. The culture medium was dark, and thymidine incorporation into acid-precipitable material was reduced to background levels (Table 2). In contrast, cells exposed to both dopa and ascorbic acid had the same morphology as control cells and the culture medium was clear. Thymidine incorporation was depressed in cells exposed to both dopa and ascorbic acid but it was significantly above background levels. Cells exposed to ascorbic acid alone appeared normal and the culture medium was clear, even though ascorbic acid reduced thymidine incorporation by about 33%. In a parallel experiment, cells were exposed to synthetic melanin (0.1 mg ml^{-1}) for 16 h. Melanin had no effect on cellular morphology and thymidine incorporation by cultures exposed to melanin was the same as that of control cultures (Table 2). Although these data do not rule out the possibility that dopa exhibited some inherent toxicity, our simplest interpretation of the results presented in Table 2 was that ascorbic acid, as long as it was present in the medium, reduced dopa quinone back to dopa and that the toxic effects were not due to dopa *per se* but rather to a compound(s) formed after dopa oxidation. As melanin itself was not toxic it seemed likely that the results were due to substances appearing after the oxidation of dopa but before the formation of melanin.

To test this interpretation, we examined the effects of 5,6-dihydroxyindole on cellular morphology and thymidine incorporation. This compound was chosen because of its ease of synthesis¹¹ and because it is only one step removed from melanin in the synthetic pathway. Cells were exposed to dopa ($2 \times 10^{-4}\text{ M}$) or 5,6-dihydroxyindole ($2 \times 10^{-4}\text{ M}$) simultaneously with ^3H -thymidine, and we found that the latter was considerably more effective than dopa in reducing the rate of thymidine incorporation into acid-precipitable material (Fig. 2). After 4 h, cells exposed to 5,6-dihydroxyindole were lysed and the culture medium was dark. In contrast, cells exposed to dopa for 4 h were not different from controls. Mouse L fibroblasts were also killed by 5,6-dihydroxyindole, indicating that this compound, in contrast to tyrosine, dopa and tryptophan, is not selectively toxic to pigment cells. A dose-response curve was constructed by comparing the effects of various concentrations

Table 2 Effects of dopa, ascorbic acid and melanin on thymidine incorporation by Cloudman S-91 cells

Additions to culture medium	^3H -Thymidine incorporation (c.p.m. per 10^5 cells per h)
None	1,694
Dopa	83
Dopa + ascorbic acid	605
Ascorbic acid	1,209
Melanin	1,630

Cells (10^5) were inoculated into Linbro multi-dish Dispo trays (2-cm^2 wells). After the cells had attached to the surface, medium was removed and fresh medium was added containing methyl- ^3H -thymidine (0.3 Ci mmol^{-1}), dopa ($4 \times 10^{-4}\text{ M}$), ascorbic acid ($1 \times 10^{-3}\text{ M}$) and melanin (0.1 mg ml^{-1} , Sigma) as indicated. After 16 h the amount of ^3H -thymidine incorporated into acid-precipitable material was determined by the following procedure. Cells were removed from the wells with EDTA (1 mM) in Ca-Mg free Tyrode salt solution, centrifuged ($2,000\text{g}$, 5 min), resuspended in NaCl (156 mM) and precipitated with an equal volume of ice-cold trichloroacetic acid (10%, containing 1 mg ml^{-1} non-radioactive thymidine). After 15 min at 4°C the acid precipitates were filtered onto $0.45\text{-}\mu\text{m}$ Millipore filters and washed with 30 ml ice-cold trichloroacetic acid (5%, containing 1 mg ml^{-1} non-radioactive thymidine). The filters were dried in air and counted in a liquid scintillation counter. Counts represent the average from 4 separate cultures and the variation was less than $\pm 15\%$. These experiments were repeated three times, with similar results each time.

of dopa and 5,6-dihydroxyindole on ^3H -thymidine incorporation (Fig. 3). About five times more dopa than 5,6-dihydroxyindole was required to achieve a 50% reduction in the incorporation of thymidine. These results suggest that 5,6-dihydroxyindole is at least one of the melanin precursors responsible for the cytotoxic effects observed after the oxidation of tyrosine and dopa by tyrosinase.

We have demonstrated that the generation of toxic products during melanogenesis is a characteristic unique to pigment cells, because tyrosinase is a gene product of these cells. Thus, tyrosine and dopa are specifically toxic to cells possessing tyrosinase activity whereas 5,6-dihydroxyindole shows a wider range of toxicity and is not specific to pigment cells. We hoped to develop these findings into a rationale for the chemotherapy of human melanoma. It is possible, however, that normal melanocytes have a protective mechanism against the toxicity of melanin precursors, as highly melanised but healthy cells are present throughout the animal kingdom and can also survive in culture for a considerable time¹². Our present goals therefore, are to identify any protective mechanism(s) involved and to understand more about the factors regulating tyrosinase activity during melanogenesis.

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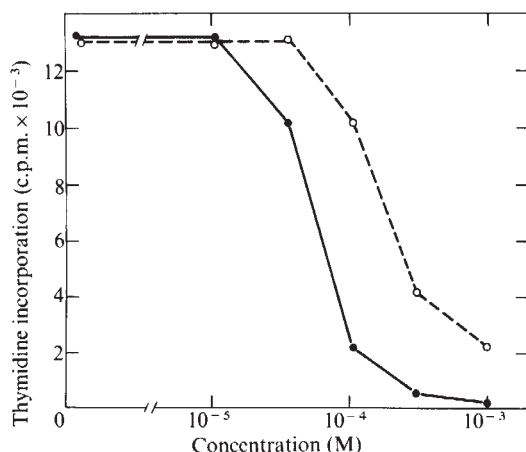
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Fig. 3 Effects of various concentrations of dopa and 5,6-dihydroxyindole on thymidine incorporation into acid-precipitable material. Cells were inoculated into Linbro wells as described in Table 2. Dopa (○) and 5,6-dihydroxyindole (●) were added at the concentrations indicated. After 4 h the amount of ^3H -thymidine incorporation into acid-precipitable material was determined in quadruplicate wells. Variation was less than $\pm 15\%$. The experiments were repeated three times, with similar results each time.



Do radiation-induced thioguanine-resistant mutants of cultured mammalian cells arise by HGPRT gene mutation or X-chromosome rearrangement?

RESISTANCE to the cytotoxic purine analogue 6-thioguanine (TG) in cultured mammalian cells is known to be associated with deficiency in the X-chromosome-linked purine-salvage enzyme, hypoxanthine-guanine phosphoribosyl transferase (HGPRT, EC 2.4.2.8). The induction of mutations to TG resistance in cultured mammalian cells has been used to quantify the mutagenic effects of various physical and chemical agents¹⁻³ and it has been argued that such mutations arise primarily as a result of true gene mutations⁴. However, because ionising radiation induces TG-resistant mutants with very low HGPRT activity⁵ and fails to induce the ouabain-resistance phenotype in mammalian cells^{6,7}, it has been suggested that ionising radiation leads to gross genetic damage rather than to point mutations in structural genes^{2,7}. Here we present evidence that gross structural changes involving the X chromosome are the genetic basis of a significant proportion of radiation-induced mutation to TG resistance in cultured human fibroblasts.

If radiation-induced mutation to TG resistance (HGPRT-deficiency) arises in cultured human fibroblasts as a consequence of gross changes to the organisation of DNA, a proportion of the more extreme changes may be visible as aberrations in the X-chromosome region containing the HGPRT locus. Gene mapping experiments have assigned the HGPRT locus in man to the terminal region of the long (q) arm of the X chromosome⁸ and using chromosome banding techniques we examined the complete karyotypes of radiation-induced TG-resistant mutants of cultured human fibroblasts so as to determine whether X-chromosome changes correlated with HGPRT deficiency.

Aberrations (exchanges and deletions) consistent with the mapped position of the HGPRT locus were apparent in the X chromosome of up to 40% TG-resistant mutants induced by various ionising radiations. Figure 1a is a summary of the pattern disruption points (PDPs) determined for 23 mutants induced by 250-kV X rays, carbon ultrasoft X rays, helium ions of 90 keV μm^{-1} , α particles from plutonium (~ 140 keV μm^{-1}) and nitrogen ions of 470 keV μm^{-1} . In the case of exchange aberrations the independence of the mutants described is clear, as all the exchanges were different. The majority of mutants with X-chromosome deletions were detected in a single experiment using nitrogen ion irradiations, and it is possible that two examples of del(X)(q26) were repeats of a single clone. With a single exception (an Xp exchange probably not associated with the mutant phenotype) all PDPs occurred in the q arm of the chromosome; from the clustering of exchange PDPs in the terminal region it may be concluded (bearing in mind the inherent 3-band uncertainty of PDP allocation⁹) that the locus determining the mutant phenotype is situated distal to Xq 22. This observation agrees with most other data on the chromosomal position of the HGPRT locus in man.

A direct association between induced TG resistance and X-chromosomal aberrations would require that such aberrations in non-mutant cells surviving the radiation should be rare. In agreement with this, we failed to detect aberrations in the X chromosomes of 25 non-mutant viable clones surviving the same dose of α particles as that used to induce the mutants. On the other hand, because the wild-type culture was of female origin the aberrations detected could be in the inactive X and unassociated with the mutant phenotype. We have not rigorously excluded this possibility, but in the case of two X-chromosome exchanges (mutants III and IV of Fig. 1b) the aberrant X chromosome completed localised DNA synthesis before the normal X (Fig. 2). Late replication is normally

associated with the inactive X and hence we conclude that in these mutants the aberrant X was the active species and was probably responsible for the mutant phenotype. Finally, it should be pointed out that techniques of mutant selection do not exclude the possibility that some mutants with X aberrations might have arisen spontaneously during post-irradiation culture. However, examination of 20 spontaneous mutant

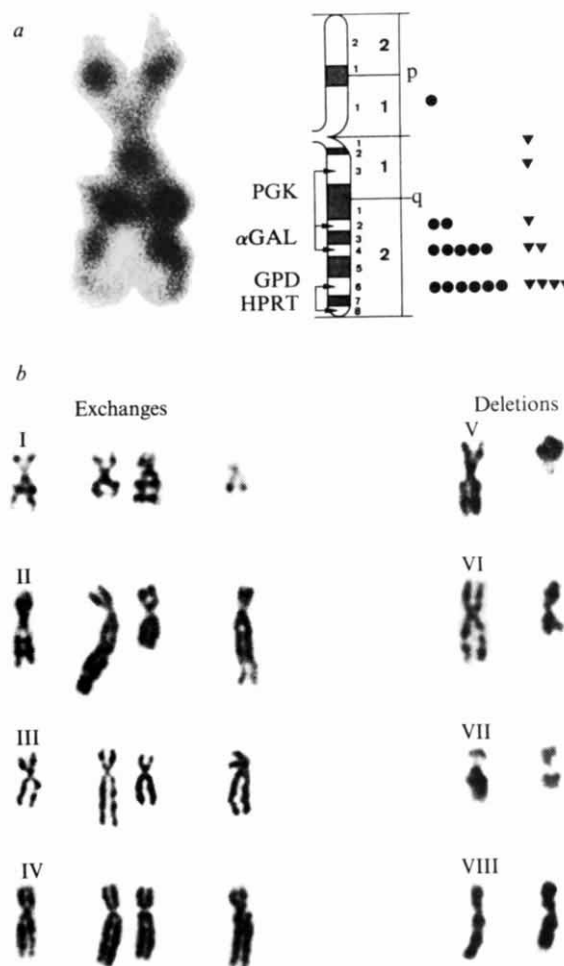


Fig. 1 a, Normal and schematic G-banded human X chromosome showing pattern disruption points detected in aberrant X chromosomes of a variety of thioguanine-resistant mutants of cultured fibroblasts induced by ionising radiations. ●, Exchange points; ▼, deletion points. Approximate positions for gene loci of PGK (phosphoglycerate kinase), α GAL (α galactosidase), GPD (glucose-6-phosphate dehydrogenase) and HGPRT (hypoxanthine-guanine phosphoribosyl transferase) are indicated. b, Examples of normal and derivative chromosomes from eight representative mutants (all 46 XX) induced by ionising radiations. Exchanges (I-IV): normal X chromosomes to the left, normal autosome to the right, derivative chromosomes central. I, t(X; 17)(q 24; q 25), mutant induced by carbon ultrasoft X rays; II, t(X; 5)(q 26; q 13), mutant induced by 250-kV X rays; III, t(X; 5)(q 24; q 13) and IV, t(X; 4)(q 26; q 31), mutants induced by α particles from plutonium. Deletions (V-VIII): normal X chromosomes to the left, deleted X to the right. V, del(X)(q 11); VI, del(X)(q 26), mutants induced by nitrogen ions of LET 470 keV μm^{-1} ; VII, del(X)(q 24), mutant induced by α particles from plutonium. (Nomenclature according to Paris Conference¹⁴). Mutants were selected as being resistant to 3 $\mu\text{g ml}^{-1}$ TG 7 d after radiation doses giving surviving fractions of 0.2 (refs 1, 15). All mutants were karyotyped using the ASG method¹⁶. The majority (including I, II and V-VIII) were karyotyped immediately after dissociation of selected mutant clones. This was a destructive technique and did not allow further study of these mutants. Six mutants induced independently by α particles were isolated and cultured before karyotyping; of these, three (III, IV and one other) showed unequivocal X-chromosome aberrations.

karyotypes failed to reveal any structural abnormalities in the X chromosomes, so such events are too rare to account for the numbers we have observed in irradiated cultures.

The conclusion that structural changes to the X chromosome result in the loss of HGPRT raises the question of whether the function of other Xq genes are also affected. As a preliminary test of this we examined the activity of glucose-6-phosphate dehydrogenase (GPD, EC1.1.1.49) using a semi-quantitative histochemical staining technique¹⁰. As judged by staining intensity, the variations in GPD activity among α -particle-induced TG-resistant clones was considerably greater than that observed among non-mutant surviving clones, with up to 5% of mutant clones showing very low activity and a similar number showing super-activity. In electrophoretic enzyme assays (Fig. 3) two karyotypically defined Xq exchange mutants were completely lacking in HGPRT but were not obviously deficient or super-active in GPD. However, because almost all mutant clones showing atypical GPD activity in histochemical assays showed very poor growth it has not yet been possible to carry out truly representative karyotypic and electrophoretic analyses, and hence the question of multiple gene effects in radiation-induced mutants remains unanswered.

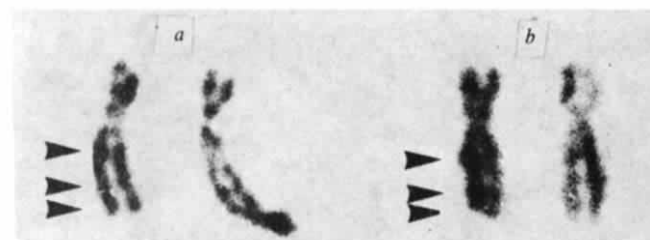


Fig. 2 Late replication of normal X-chromosome regions in two α TG-resistant mutants with X-chromosome aberrations. *a*, Mutant III of Fig. 1*b*; *b*, mutant IV of Fig. 1*b*. For each chromosome pair the normal X is to the left. Late replicating regions of chromosomes were detected using the 5-bromodeoxyuridine (BUDR)-thymidine-33258 Hoechst method of Grzeschnik, Kim and Johannsman¹⁷ with the modification that Jenner stain was used in place of Giesma. With this method late replication of a chromosome region is characterised by dark staining (indicated by arrows); early replicating regions (which incorporate BUDR into DNA) show lighter staining because the reaction of 33258 Hoechst with BUDR-substituted DNA causes that DNA to bind less Giesma or Jenner stain. The lighter staining of the q bands of the aberrant X chromosomes indicate that they were replicated early in the cell cycle.

From the karyotypic analysis presented in Fig. 1*b* it is possible to explain the TG-resistance phenotypes of radiation-induced mutants without invoking structural gene mutations. In the case of the deletion mutants (Fig. 1*b*, V–VIII), TG resistance probably arises by complete loss of the HGPRT gene from the karyotype in the acentric fragment which resulted from an incomplete radiation-induced structural aberration. For the exchange mutants (Fig. 1*b*, I–IV), loss of HGPRT could result from gene inactivation as a consequence of position effect¹² or, alternatively, the apparently simple reciprocal exchange configuration observed in mutants many generations after induction may be the stable manifestation of a more complex initial exchange event resulting in the loss of the HGPRT gene in a small interstitial chromosome deletion. The relatively poor resolution obtained with karyotypic analysis means that many small Xq changes will be undetected. It is therefore possible that the majority of radiation-induced TG-resistant mutants of cultured human fibroblasts arise by a process involving changes in chromosome structure rather than by true gene mutation. These conclusions on the nature of radiation-induced mutations in cultured mammalian somatic cells agree generally with *in vivo* observations on radiation-induced mutations in mouse germinal cells, many of which show phenotypic characteristics consistent with chromosomal changes¹³.

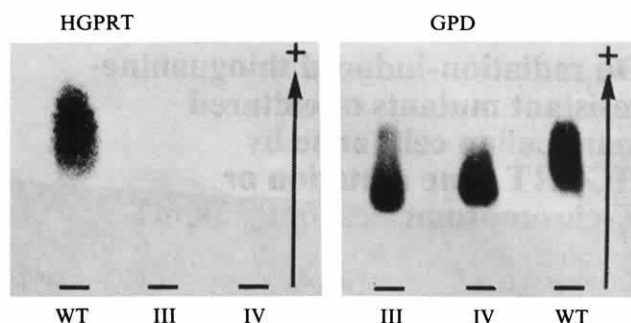


Fig. 3 Cellulose acetate gel electrophoresis of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and glucose-6-phosphate dehydrogenase (GPD) in extracts of wild-type and TG-resistant mutants of cultured human fibroblasts. WT, wild type; III and IV refer to the exchange mutants of Fig. 1*b*. Cells (2×10^6) washed twice with isotonic salts solution were lysed for 15 min at 0°C in 0.1 ml Tris-HCl buffer (50 mM), pH 8.0, 0.1% Triton. Cell debris was sedimented by centrifugation at 1,000 r.p.m. for 10 min at 0°C; 5- μ l samples of the supernatant were used for each enzyme determination. Cellulose acetate gel plates (60 $\times$ 75 mm), applicators and electrophoresis chambers were all Zip Zone (Helena). The methods were as follows. HGPRT: electrophoretic buffer, Tris-citrate (15 mM), pH 7.6; separation at 200 V for 45 min with ice cooling. Reaction mixture: Tris-HCl buffer (50 mM), pH 7.4, 2.0 ml; ¹⁴C-hypoxanthine (25 μ Ci ml⁻¹), 0.3 ml; MgCl₂ (0.25 M), 0.4 ml; phosphoribosyl pyrophosphate (0.01 M), 0.3 ml. A DEAE paper overlay method with subsequent autoradiography¹⁸ was used to determine the location of the ¹⁴C reaction product (inosine monophosphate). GPD: electrophoretic buffer, Tris-glycine, pH 8.5 (3.0 g Tris, 14.4 g glycine w/w per l); separation at 200 V for 40 min with ice cooling and 2 μ g NADP added to the sample to stabilise GPD. Reaction mixture: Tris-HCl buffer (50 mM), pH 8.0, 1.0 ml; MgCl₂ (0.25 M), 0.1 ml; NADP (1 mg ml⁻¹), 1.0 ml; phenazine methosulphate (2.5 mg ml⁻¹), 0.1 ml; monotetrazolium (10 mg ml⁻¹), 0.1 ml; glucose-6-phosphate (0.5 M), 0.2 ml. The staining procedure was to mix the reaction solution with 2 ml molten aqueous agarose (1.4%) at 60°C and then pipette over the gel plate. The tetrazolium reaction was allowed to proceed for 20 min at 37°C in darkness, after which the agar was washed from the plate and the plate subsequently washed and dried.

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Competition of normal β chains and sickle haemoglobin β chains for α chains as a post-translational control mechanism

ADULT human haemoglobin (HbA) is a tetrameric protein with two α and two β polypeptide chains. Theoretically, individuals heterozygous for a β -chain mutation should have equal amounts of the normal HbA ($\alpha_2\beta_2^A$) and an abnormal variant ($\alpha_2\beta_2^S$) in their red cells. However, these people usually have different amounts of the two haemoglobins. For example, the red cells of sickle cell trait individuals usually contain about 60% HbA and 40% HbS¹⁻⁵. Those who also have an α -thalassaemia gene(s), which decreases production of α chains, have an even lower proportion of HbS⁶⁻¹². Each of these two haemoglobin polymers has an α -chain type common to both proteins as well as a specific β^A or β^S chain type. The individual α , β^A , and β^S chain types can be isolated *in vitro* in a functional form so that the haemoglobin tetramers can be reconstituted from appropriate mixtures of α and β chains¹³. Here we report that more HbA than HbS was formed when a mixture of equal amounts of β^A and β^S chains was incubated with limiting concentrations of α chains. These results suggest that a similar form of post-translational control of haemoglobin assembly may exist in some erythroid cells *in vivo*. The accomplishment of these experiments and the interpretation of the results were greatly facilitated by the use of radiolabelled haemoglobin chains of high specific activity.

Red blood cells were obtained from a normal human adult and from an individual with sickle cell anaemia. The sickle blood cells were incubated with ^{14}C -leucine in a mixture supporting protein synthesis¹⁴, to provide a source of ^{14}C -labelled β^S chains. Haemolysates of both normal and sickle cells were treated with *p*-chloromercuribenzoate, which reacts covalently with protein sulphhydryl groups, to dissociate the haemoglobins into the haem-containing subunit α and β chains¹³. The β^A chains were isolated from the normal haemolysate by the procedure of Geraci *et al.*¹⁵, and the ^{14}C -labelled β^S chains were isolated from the sickle haemolysate by the modification of McDonald and Noble¹⁶ using a column of DEAE-Sephadex A50. α Chains were prepared with a ^3H label so that the relative amounts of HbA and HbS formed in the reaction with β chains could be measured quantitatively. To provide α chains of high specific radioactivity, blood reticulocytes with unusually high haemoglobin synthetic activity were obtained from an individual with Hb Sabine, a β -chain variant, and were incubated with ^3H -leucine¹⁷. The Hb Sabine was isolated from the haemolysate by electrophoresis in columns of polyacrylamide gel, and normal, ^3H -labelled α chains were prepared from the abnormal haemoglobin by the method of Geraci *et al.*¹⁵.

To form haemoglobin tetramers in physiological conditions, it was necessary for the isolated α and β chains to be entirely free of mercuribenzoate. This was accomplished by treatment of the mercurated chains with mercaptoethanol to regenerate the sulphhydryl groups, as described elsewhere^{15,16}. We found less than 0.01 mol of Hg for each mol of chain when our preparations were analysed for the presence of residual Hg by atomic absorption spectrophotometry. All isolated chains were dialysed against 0.01 M potassium phosphate, pH 7.0, and stored at 4 °C under an atmosphere of carbon monoxide.

Various amounts of ^3H -labelled α chains were added to replicate mixtures containing excess, but equal, amounts of β^A and ^{14}C -labelled β^S chains. The reaction mixtures were incubated at 4 °C for 60 min, sufficient time for the isolated α and β chains to react completely and form Hbs A and S. The haemoglobin products and excess chain reactants were separated by electrophoresis of a sample of each reaction mixture on a strip of cellulose acetate. The distribution of ^3H radioactivity among the various protein components was

determined by analysis of sections of the strip in a liquid scintillation system¹⁴.

Figure 1 shows the results from a reaction in which the total concentration of β chains was eight times greater than that of α chains. About 40% of the total ^3H radioactivity migrated as HbS whereas 60% migrated as HbA. These data indicate that more α chains reacted with β^A chains to form HbA than with β^S chains to form HbS. In a control experiment, unreacted free α chains migrated in this electrophoretic system near the line of application at a position well separated from the haemoglobin tetramers (data not shown). The use of ^3H -labelled α chains allowed the precise detection of excess β chains. Most of the ^{14}C -labelled β^S chains migrated slightly anodal to HbA in a position characteristic of unreacted β^S chains. As expected, a small fraction (about 10%) of the ^{14}C -labelled β^S chains migrated as HbS (Fig. 1). The high (>90%) recovery of β^S chain ^{14}C radioactivity in these two components eliminates selective instability or loss of β^S chains as explanations for the formation of less HbS than HbA.

Figure 2 shows the results from several reactions in which various concentrations of α and β chains were used. In each experiment, the initial amount of β^A chains was equal to that of ^{14}C -labelled β^S chains. The amount of HbS formed was less than that of HbA in all experiments in which α chains were present in limiting amounts ($\alpha/\text{total } \beta \text{ ratio} < 1$). In these experiments, essentially all the ^3H -labelled α chains reacted to form HbA or HbS. Conversely, when α chains were present in an amount equal to or greater than that of total β chains, the amount of HbS formed was similar to that of HbA. More than 90% of the available ^3H -labelled α chains reacted to form Hbs S and A when the initial concentration of α chains was identical to that of total β chains. As expected, about half (47%) the ^3H -labelled α chains reacted to form the two haemoglobins in equal amounts when the initial concentration of α chains was twice that of total β chains. These results indicate that essentially all the β^A and β^S chains were able to combine with the complementary α chains in the reaction mixtures. Thus, our observation of a decreased production of HbS with limiting concentrations of α chains cannot be due merely to the existence of a fraction of β^S chains unable to react with the α chains.

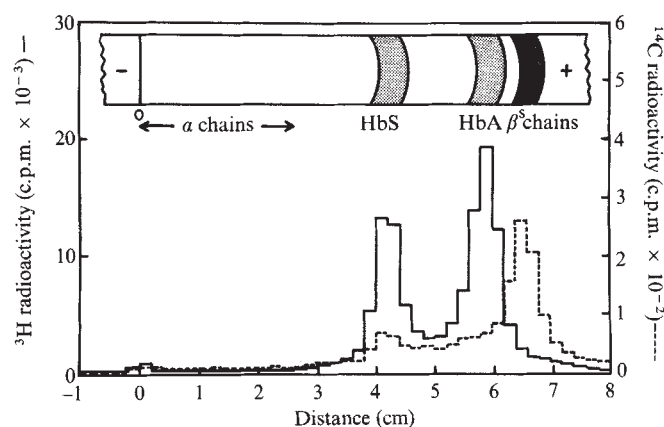


Fig. 1 Pattern of protein radioactivity after electrophoresis of a reaction mixture of α , β^A , and β^S chains. The mixture had 62 μg of ^3H -labelled α chains and 246 μg each of β^A and ^{14}C -labelled β^S chains in a total volume of 0.040 ml of 0.01 M potassium phosphate, pH 7.0. After incubation for 60 min at 4 °C, a sample of 5 μl of the reaction mixture was applied to a cellulose acetate strip. The electrophoresis was done, and the strip was cut into sections for radioactivity analysis, as described previously¹⁴. The positions of the stained bands of product haemoglobins and residual reactant chains are indicated at the top of the figure, and the line of sample application is denoted by zero. The β^A chains migrated off the anodal end of the strip during the electrophoresis. Almost 100% of the ^3H - and 91% of the ^{14}C -radioactivities applied to the strip were recovered in the sections.

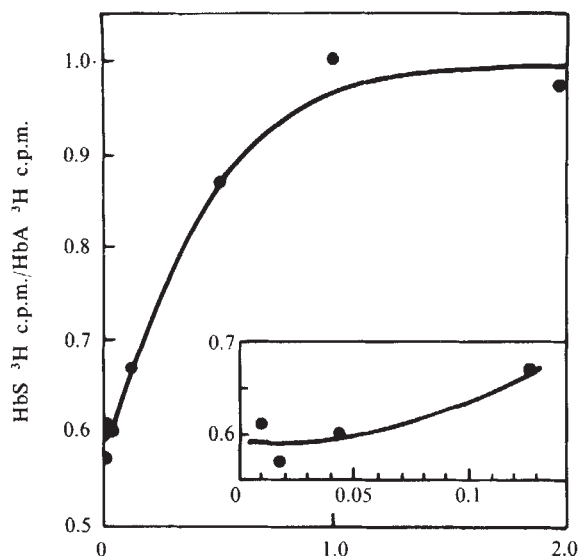


Fig. 2 Comparison of product haemoglobin radioactivities with the relative, initial concentrations of reactant chains for several experiments similar to that described in the legend to Fig. 1. The HbS/HbA ^3H -radioactivity ratio represents the relative amounts of ^3H radioactivity detected in the two haemoglobins after electrophoresis. The $\alpha/(\beta^A + \beta^S)$ chain ratio represents the relative initial amounts of α and total β chains. The inset shows the results from four experiments in greater detail. There were initially 246 μg each of β^A and β^S chains in these four reaction mixtures, with $\alpha/(\beta^A + \beta^S)$ chain ratios < 0.2 . The three reaction mixtures with $\alpha/(\beta^A + \beta^S)$ chain ratios of 0.5, 1.0 and 2.0 had initially 49, 25 and 12.5 μg , respectively, of each type of β chain in a total volume of 0.040 ml.

The ratio of HbS to HbA formed in these reactions fell with decreasing initial relative α -chain concentration (Fig. 2). However, when the relative α -chain concentration was sufficiently low ($\alpha/\text{total } \beta$ ratio < 0.05), the ratio of formation of HbS to HbA remained constant at about 0.6 (Fig. 2, inset). These HbS/HbA ratios represent amounts detected after complete reaction of the reagent chains during the 60-min incubation period. Although these were equilibrium measurements, the limiting value of about 0.6 for this ratio probably reflects the rates at which individual β^S and β^A chains reacted with α chains. When the α -chain concentration was increased, the resulting HbS/HbA ratio went from 0.6 to unity, as enough α chains were now available to combine with both β^S and β^A chains.

The β -chain monomer is probably the molecular species which reacts with α chains. As implied above, the difference in amounts of HbS and HbA formed in these studies probably occurs because of a difference in the relative affinities of β^S and β^A chain monomers for α chains. However, the following alternative explanation for our results remains to be eliminated. β Chains prepared by methods similar to those used in the present work exist almost exclusively in the tetrameric form, even at concentrations 50-fold lower than those used in our experiments¹⁸⁻²³. The difference in amounts of HbS and HbA formed could conceivably be caused by a difference in the initial concentrations of β^S and β^A monomers resulting from unequal rates of dissociation of β_4^S and β_4^A tetramers rather than by a difference in affinities of β -chain monomers for α chains.

A similar difference in affinities of β^S and β^A chains for α chains was reported by Abraham and Huisman²⁴ and more recently by Lee *et al.*²⁵. However, in their experiments, mercuribenzoate, added to cause tetramer dissociation during chain preparations, was still covalently bound to the subunits. Following mixture of the mercurated α and β chains, mercaptoethanol was added to aid the reconstitution of the haemoglo-

bin tetramers by removal of the mercurial reagent. The direct addition of mercaptoethanol may result in the denaturation of a significant fraction of the chains. In our work, these denatured chains were removed during the preparative procedures. Moreover, we have found that, when partially mercurated chains are present in the reaction mixture, reconstitution of native haemoglobin is less efficient, and the tetramers formed migrate in a heterogeneous manner during electrophoresis. It is conceivable that the presence of the rather bulky mercury groups induces spurious recombination of α and β chains and influences the relative amounts of HbS and HbA observed. In the present work, the complete removal of the mercurial reagent before reaction of the chains ensured the reconstitution of haemoglobins in physiological conditions. Moreover, the use of ^3H - and ^{14}C -labelled chains enabled us to detect any chains partially damaged in preparation and therefore unable to form proper haemoglobins.

The results of our experiments suggest a type of post-translational control which may occur in the synthesis and assembly of HbA and HbS in some sickle trait erythroid precursor cells. Past studies have shown that the reticulocytes of sickle trait individuals who carry an α -thalassaemia gene have a lower rate of synthesis of α chains than of β chains^{26,27}. These individuals also have an unusually low concentration of HbS (about 25–30% HbS, 70–75% HbA) in their red cells. A limiting concentration of newly synthesised α chains in the reticulocytes and other red cell precursors may contribute to the decreased concentration of HbS by a mechanism similar to that observed in these experiments. An analogous model has previously been proposed to explain the different concentrations of HbH and Hb Bart's observed in individuals with HbH disease^{28,29}, an α -thalassaemia disorder. Conversely, β -chain competition for α chains probably does not contribute to the difference in HbS and HbA levels in sickle trait individuals without an α -thalassaemia gene(s) and without the concomitant deficiency in reticulocyte α -chain synthesis^{26,27}.

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Transfer of the drug-resistance transposon Tn5 to *Rhizobium*

TRANSPOSONS are discrete sequences of DNA that are incapable of self-replication and can insert into DNA replicons, such as chromosomes or plasmids, in the absence of the *recA* gene function¹. Insertion can be random and where it occurs into the continuity of a gene leads to non-leaky polar mutations¹. Some transposons carry drug resistance genes and thus insertions can be mapped as drug-resistance markers¹. We report here that Tn5, a transposon coding for kanamycin resistance², can insert into the chromosome of *Rhizobium leguminosarum*, the bacterium responsible for nodulation and nitrogen fixation in *Pisum*, *Vicia*, *Lathyrus* and *Lens*.

P1 group R plasmids, such as RP4, are able to infect most Gram-negative bacteria^{3,4} and have been valuable for establishing linkage maps for many species^{5–8}. Transfer between *Escherichia coli* and *Rhizobium* usually occurs at a frequency of about 10^{-2} (ref. 5). Recently Boucher *et al.*⁹ reported that when the lysogenic phage Mu was inserted into RP4 this frequency was reduced about 10^5 -fold and the plasmids that were transferred were altered. Van Vliet *et al.*¹⁰ have since shown that the same applies to transfers from *E. coli* to *Agrobacterium tumefaciens* and that those plasmids which do become established in this new host have large deletions of the Mu phage. Restriction and the functioning of at least one Mu gene (*r23*) are both involved in reducing the ability of these plasmids to become established^{9,10}. Van Vliet *et al.*¹⁰ also reported that the inability of RP4::Mu plasmids to become established in *A. tumefaciens* could be utilised to carry transposons into this species to obtain transposon mutagenesis. Tn7 insertion and deletion mutations in *A. tumefaciens* plasmids have since been important in the genetic analysis of the role of such plasmids in tumour formation¹¹.

We attempted to use an RP4::Mu::Tn7 plasmid (provided by Dr M. van Montagu, Gent) to obtain transposon mutagenesis in *R. leguminosarum*. Transfer of either the RP4 or Tn7-coded drug resistances from an *E. coli* donor could not be detected (frequency less than 10^{-8}) in crossing conditions in which the frequency of wild-type RP4 transfer was 10^{-2} . Although these crosses were unsatisfactory for introducing Tn7 into *R. leguminosarum* they demonstrated that Mu insertions into RP4 could be used to prevent establishment of this plasmid in this host. Tn5 (kanamycin) and Tn10 (tetracycline) are probably the best studied transposons¹ and both are relatively nonspecific in their sites of integration into the *E. coli* chromosome¹. As RP4 codes for resistance to both these antibiotics we constructed a new P1 plasmid carrying Mu (see footnote to Table 1), using a plasmid that coded only for gentamicin, spectinomycin and low-level streptomycin resistance (pPH1JI)¹². A derivative of this plasmid carrying Mu and Tn5, called pJB4JI, was used as a carrier of Tn5 into three *Rhizobium* species (Table 1). The data in Table 1 show that the ability of pJB4JI, to become established in these species was reduced relative to the parent plasmid pPH1JI. Bacteria resistant to kanamycin, kanamycin plus gentamicin and gentamicin alone were found in these crosses (Table 1). None of these was found to be able to transfer drug resistance to *E. coli* (frequency less than 10^{-8}) in conditions

where pPH1JI transfer occurred at a frequency of approximately 10^{-2} .

To be useful as a generalised mutagen a transposon needs to be relatively nonspecific in its insertion. This was tested for Tn5 by replica-plating kanamycin-resistant derivatives selected on complete medium to minimal medium and scoring for auxotrophy. For *R. leguminosarum*, *R. trifolii* and *R. phaseoli* the frequency of auxotrophy was about 0.3% (compared to a spontaneous rate of less than 10^{-8}) and the range of auxotrophs found suggests that insertion was random. Auxotrophic mutants with the following requirements have been found to date: for *R. leguminosarum*, leucine (2), adenine (5), serine (2), riboflavin (1), methionine (2), adenine or guanine (1), phenylalanine + tyrosine (1) and two with unknown requirements; for *R. trifolii*, tryptophan (2), adenine or guanine (1), leucine + isoleucine + valine (1); for *R. phaseoli*, phenylalanine (1), tryptophan (2), methionine (1), leucine (1), adenine (2), uracil (1), cysteine or methionine (2) and two with unknown requirements. These results indicate the potential for obtaining apparently random mutagenesis in *Rhizobium* species.

Because the vehicle for introducing Tn5 into *Rhizobium* carried phage Mu, it is conceivable that the mutagenesis was

Table 1 Transfer of kanamycin (Km) and gentamicin (Ge) resistance from *Escherichia coli* to *Rhizobium* species*

Recipient	pPH1JI	Plasmid in donor†		
		pPH1JI::Mu::Tn5 (pJB4JI)		
	Ge	Ge	Km	Ge + Km
<i>R. leguminosarum</i> 726	10 ⁻²	10 ⁻⁷	10 ⁻⁶	5 × 10 ⁻⁸
<i>R. trifolii</i> 6709	10 ⁻¹	10 ⁻⁶	5 × 10 ⁻⁶	2 × 10 ⁻⁸
<i>R. phaseoli</i> 1234	10 ⁻¹	10 ⁻⁶	5 × 10 ⁻⁶	1.5 × 10 ⁻⁸

Crosses were performed as described by Beringer *et al.*⁵ except that mating was allowed to proceed for only 4 h at 28°C. The transfer of plasmids and resistance markers was selected on complete (TY)⁵ medium supplemented with gentamicin ($5 \mu\text{g ml}^{-1}$), kanamycin ($20 \mu\text{g ml}^{-1}$), streptomycin ($50 \mu\text{g ml}^{-1}$) and rifampicin ($20 \mu\text{g ml}^{-1}$) as required. The transfer of gentamicin plus kanamycin resistance was determined by replica-plating colonies selected on a single antibiotic to medium containing the other. *R. leguminosarum* strain 726 is a rifampicin resistant derivative of our wild-type strain 300 (ref. 15). *R. trifolii* strain 6709 and *R. phaseoli* strain 1234 are streptomycin-resistant derivatives of strains 204 and 3644 from the Rothamsted Culture Collection. Plasmid pJB4JI was constructed in this laboratory by selecting Mu⁺ lysogens of an *E. coli* J53 strain (carrying the P1 group plasmid pPH1JI) after spotting Mu on to a lawn of log phase bacteria. Four lysogens were crossed to a Mu *cts* lysogen of *E. coli* (strain SB1801) with selection for gentamicin resistance carried on pPH1JI and growth at 42°C. Gentamicin-resistant colonies growing at 42°C were presumed to carry pPH1JI and the wild-type Mu which was required to suppress the thermoinducible Mu *cts* phage in strain SB1801. Ten clones were picked and regrown under these selective conditions before crossing with an *E. coli* J53 *nal* strain and separately with an *R. leguminosarum* recipient in patch matings as described by Johnston, Setchell and Beringer¹⁶. All 10 clones transferred gentamicin resistance and the ability to produce Mu phage to strain J53 *nal*; none transferred gentamicin resistance to the *R. leguminosarum* strain, though transfer of pPH1JI would have occurred. Each clone was assumed to carry Mu inserted into a part of the plasmid not carrying important transfer genes. One clone was crossed into an *E. coli* recipient (strain 1752) carrying a chromosomally inserted Tn5. The pPH1JI::Mu derivative of strain 1752 was crossed with a suitable *E. coli* recipient with selection for kanamycin transfer. Kanamycin-resistant transconjugants were crossed to a J53 *nal* recipient and a kanamycin and gentamicin-resistant derivative (strain 1830) was purified. Strain 1830 transferred both resistances at high frequency between *E. coli* strains and was found to be Mu immune but unable to produce viable Mu phage. This indicates that Tn5 had inserted either into Mu or beside it, causing a deletion into Mu. The pPH1JI derivative plasmid carrying Mu and Tn5 is called pJB4JI and the pPH1JI::Mu parent plasmid is called pJB9JI.

* Frequencies of transfer are approximate and measured per recipient plated.

† Donor was *E. coli* strain J53 *nal* carrying the plasmid shown.

Table 2 Transduction of Tn5-associated auxotrophs by rhizobiophages RL38 and RL39

a Selection for the transfer of kanamycin resistance						
Donor*	Frequency of kanamycin resistance (per recipient)			Proportion of kanamycin resistant recipients also receiving Tn5-associated auxotrophy		
	RL38	RL39	Control	RL38	RL39	Control
T94	1×10^{-5}	1×10^{-6}	1×10^{-8}	100/100	95/95	0/4
T95	8×10^{-6}	1×10^{-6}	1×10^{-8}	95/95	64/64	0/1
T133	6×10^{-6}	9×10^{-7}	$< 10^{-8}$	250/250	150/150	—

b Selection for the transfer of prototrophy						
Recipient	Frequency of prototrophy (per recipient)			Proportion of prototrophic recipients also kanamycin sensitive		
	RL38	RL39	Control	RL38	RL39	Control
T94	2×10^{-5}	7×10^{-7}	$< 10^{-8}$	100/100	100/100	—
T95	3×10^{-5}	2×10^{-6}	5×10^{-8}	292/292	249/251	3/7
T133	2×10^{-6}	3×10^{-7}	3×10^{-8}	544/545	284/284	10/10

* T94, 95 and 133 are derivatives of the *R. leguminosarum* strain 300 carrying respectively auxotrophic requirements for adenine, leucine and arginine + uracil (pyrA?) resulting from the transfer of Tn5 from *E. coli*. Phages RL38 and RL39 have recently been isolated as lytic phages for *R. leguminosarum* strain 300 (A.V.B.-W., in preparation). Transduction was essentially as described by Ely and Johnson¹⁷ for *Caulobacter crescentus*. Phages were irradiated with ultraviolet light to 0.1% survival and added to the recipient at a multiplicity of infection of about one phage per bacterium. Suitably marked strains of *R. leguminosarum* were used in these crosses so that contamination of phage preparations by viable bacteria would have been detected in the analysis of recombinants.

associated with Mu insertions into the chromosome¹³. This is unlikely for a number of reasons.

(1) Van Vliet *et al.*¹⁰ have reported that a major factor limiting the ability of RP4::Mu plasmids to become established in *A. tumefaciens* is likely to be the formation of extensive deletions in the plasmid, which do not occur in the absence of Mu. Those few plasmids that do become established have extensive deletions of Mu. Thus the inheritance of fully functional Mu genomes would appear to be unlikely.

(2) A direct association between kanamycin resistance and induced auxotrophy was demonstrated by transduction (Table 2) and conjugation (J.E.B. unpublished results). The data for transduction are particularly convincing as relatively small pieces of DNA are transferred. They show an almost complete correlation of auxotrophy with resistance, whether selection was made for prototrophy or for transfer of kanamycin resistance (Table 2).

(3) Twelve auxotrophic mutants arising from the transfer of Tn5 were tested for reversion to prototrophy. For seven of these mutants, reversion to prototrophy was not detected (frequency less than 10^{-8}) and for two others, occasional colonies of presumptive revertants were detected (frequency about 10^{-8}). Three strains had reversion rates of about 10^{-8} , 10^{-5} and 10^{-6} , and respectively 10%, 34% and 90% of revertants were kanamycin sensitive. The genetic basis of the reversions observed was not determined, but it can be assumed that the kanamycin-sensitive revertants were not due to suppression but to a precise excision of the insertion which had caused the mutation. Spontaneous reversion of Mu-induced mutations in *E. coli* is exceedingly rare¹³, which suggests that at least the reverting mutants were not Mu-induced, unless the precise excision of Mu from the *R. leguminosarum* chromosome is more frequent than from the chromosome of *E. coli*.

This work clearly shows that a plasmid carrying Mu can be used to introduce a transposon (Tn5) into three *Rhizobium* species. This transfer is associated with the formation of auxotrophic mutants in these species and has been used to provide a readily selectable phenotype for at least one indigenous plasmid in *Rhizobium*¹⁴. We cannot be certain, at present, that Mu is not involved, but this does not detract from the value of the technique for establishing mutagenesis by an easily mapped

insertion 'mutagen'. This should be of particular value in studies of the *Rhizobium*-legume symbiosis as the analysis of defective mutants of *Rhizobium* depends on plant tests. Thus the mapping of symbiotically-defective mutants obtained by 'conventional' means requires that each recombinant from a cross be tested individually on a plant. However transposon-induced mutations can be mapped by mapping the position of the drug resistance carried on the transposon. Therefore transposon-induced symbiotic mutations can theoretically be mapped using the minimum of plant tests, which are expensive and time consuming.

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High frequency transfer of nodulating ability between strains and species of *Rhizobium*

THE most striking and important feature of members of the genus *Rhizobium* is their ability to nodulate and fix nitrogen in the roots of legumes. There is specificity in this interaction. Legumes belonging to one cross-inoculation group are nodulated only by certain classes of *Rhizobium* and indeed *Rhizobium* species are defined in terms of the host legumes that they can nodulate—for example, *R. leguminosarum* nodulates peas, *R. trifolii* nodulates clover and *R. phaseoli* nodulates beans of the genus *Phaseolus*. The genetic bases of the nodulation process and of host specificity are poorly understood. Many *Rhizobium* strains harbour plasmids¹⁻⁴ and genetic evidence has suggested that such plasmids might carry some of the information required for the development of the symbiosis^{5,6}. We report here that the ability to nodulate peas can be transferred at high frequency from a strain of *R. leguminosarum* to a non-nodulating strain of *R. leguminosarum* and to three other species of *Rhizobium* that nodulate legumes other than peas. We infer from this that some

of the genetic information required for nodulation is plasmid-linked.

In the previous paper⁷ the insertion of the transposon Tn5 (conferring kanamycin resistance) into the chromosome of strains of *Rhizobium* was described. Using the same method we attempted to insert Tn5 into pRL1JI, a conjugative plasmid first found in an *R. leguminosarum* field isolate (strain 248). Strain 248 produces a bacteriocin and this ability can be transferred at high frequency ($\sim 10^{-2}$) to other strains of *Rhizobium*⁸. pRL1JI was transferred by conjugation into *R. leguminosarum* strain 1062 (*ura14 trp16 str69*), a derivative of strain 300 (ref. 9). One pRL1JI transconjugant, strain 2406, was used as a recipient in a cross with *Escherichia coli* strain 1830 that carries pJB4JI (ref. 7) (pPH1::Mu::Tn5) and selection was made for the transfer of kanamycin resistance. Resistant colonies arose at frequencies of $\sim 10^{-6}$ and 20 of them were used in patch crosses with *R. leguminosarum* and separately with *E. coli* recipients. None transferred kanamycin resistance to *E. coli* but one transferred it at very high frequencies to *R. leguminosarum*. This strain (T3) and a control that did not give kanamycin transfer in the patch crosses (T10), were each crossed with *E. coli* and *R. leguminosarum* using membrane crosses to determine frequencies of transfer. None transferred kanamycin resistance to *E. coli* (frequency $< 10^{-8}$). T3 and T10 transferred kanamycin resistance to the *R. leguminosarum* recipient at frequencies of about 10^{-2} and 10^{-8} per recipient, respectively. Recipients of kanamycin resistance from T10 may have arisen by the pick-up of Tn5 onto a conjugative plasmid.

Strain T3 failed to produce the bacteriocin specified by pRL1JI; one possible explanation was that Tn5 had been inserted into (or beside) the bacteriocin production gene. To test this hypothesis T3 was crossed with a strain of *R. leguminosarum* that did not produce the bacteriocin. One kanamycin-resistant transconjugant was purified and was used to propagate the transducing phage RL38 (A.V.B.-W., in preparation). The lysate was used to transduce a strain of *R. leguminosarum* that carried pRL1JI and hence produced bacteriocin. Kanamycin-resistant transductants arose at a frequency of $\sim 10^{-6}$ per recipient cell; 99.5% of them failed to produce bacteriocin, showing that kanamycin resistance was closely linked to the inability to produce bacteriocin and suggesting that Tn5 had indeed inserted into pRL1JI and had caused the loss of production in strain T3.

Strain T3 was used as a donor in crosses with the strains in Table 1. In all cases kanamycin-resistant transconjugants arose at frequencies of $\sim 10^{-2}$. When each of the parental strains was plated separately on the selective media colonies occurred at frequencies of $\sim 10^{-8}$. From each cross several transconjugants as well as control clones of the recipient strain which had not acquired kanamycin resistance were used to inoculate peas.

The control clones nodulated peas rarely or not at all (Table 1). One plant inoculated with one clone of strain 6710 had a single small nodule. Such sporadic nodulation of peas by *R. trifolii* has been observed before¹⁰. In contrast, the transconjugants induced many nodules per plant, showing that the ability to nodulate peas could be transferred to a non-nodulating strain of *R. leguminosarum* and to other species of *Rhizobium*. Most of the *R. trifolii* and *R. phaseoli* transconjugants began to produce nodules about one week later than normal and the nodules were smaller than those found following inoculation of peas by *R. leguminosarum*. Strain 9009 is a slow-growing strain able to nodulate *Cicer*. The first appearance of nodules formed by transconjugants of this strain was delayed for a further 4–5 d and, in contrast to the other recipients, not all of them nodulated peas; two failed to do so. However all three plants inoculated by one of the *R. phaseoli* transconjugants had nodules that appeared at the normal time and were of similar size and number to those found following inoculation by *R. leguminosarum*.

The roots of a sample of the plants inoculated by transconjugants were tested for acetylene reduction as an assay for nitrogenase activity (Table 1). All the strain 6015 transconjugants were able to reduce acetylene, but the interspecific

transconjugants varied in their ability to do so. All of the *R. phaseoli* transconjugants, but only a minority of those of the other two species, reduced acetylene and with a single exception the rates were low ($\sim 10\%$ of those found following inoculation of peas by strains of *R. leguminosarum*). The exception was the *R. phaseoli* transconjugant that nodulated peas as well as the *R. leguminosarum* control; this reduced acetylene at normal rates. It is not clear why the ability to reduce acetylene varies both between the different recipient classes and between transconjugants of the same recipient. We cannot say whether the transconjugants which failed to reduce acetylene would have done so after prolonged growth of the plants, because in our conditions of growing peas the plants became distressed about five weeks after germination.

The *R. trifolii*, *R. phaseoli* and strain 9009 transconjugants were used to inoculate *Trifolium repens* (var. S100), *Phaseolus vulgaris* (var. Fino) and *Cicer arietinum* L. (var. G130) respectively, these being the normal hosts for the parents of these transconjugants. Chickpeas (*C. arietinum*) were inoculated and grown in the same way as peas; the methods used for beans and clover were essentially as have been described⁹ except that clover seedlings were grown in vertically held Petri dishes (B. Rolfe, personal communication). All the interspecific transconjugants formed nodules on their original hosts but the nodules were slow to appear and were fewer and smaller than those induced by their respective parents on the same hosts. Interestingly, of the *R. phaseoli* transconjugants tested, the one that best nodulated peas was poorest on beans, inducing only a single ineffective nodule on a total of three inoculated plants.

The finding that the ability to nodulate peas was transferred from a strain of *R. leguminosarum* at high frequency is strong evidence that in this species there is a plasmid carrying genetic information required for nodulation. We cannot say whether this information is in the bacteriocinogenic plasmid pRL1JI or in another plasmid that was cotransferred at high frequency. T3

Table 1 Transfer of ability to nodulate peas and reduce acetylene in the nodules from strain T3 to other strains of *Rhizobium*

Recipient strain	Species	Markers	Control clones	Transconjugants clones	
			No. nodulating/ no. tested	No. nodulating/ no. tested	No. reducing C ₂ H ₂ / no. tested
6015	<i>R. leguminosarum</i>	<i>phe1</i> <i>trp12 rif</i> + 392 <i>str37 inf</i>	0/9	17/17	8/8
6661	<i>R. trifolii</i>	<i>rif</i>	0/7	16/16	2/9
6710	<i>R. trifolii</i>	<i>rif</i>	1/10	17/17	1/8
1233	<i>R. phaseoli</i>	<i>rif</i>	0/6	9/9	9/9
9009	<i>R. 'species'</i>		0/10	8/10	2/8

Strain 6015 is a non-nodulating derivative of strain 897 (ref. 14) isolated following ultraviolet treatment. In many previous studies it had never been shown to nodulate peas. Strains 6661, 6710 and 1233 are *rif* mutants of strains 12, 204 and 3644 respectively of the Rothamsted Culture Collection. Strain 9009 is strain 3889 from Rothamsted; it is a member of the cowpea miscellany. These last four strains were effective (that is, reduced acetylene) on their hosts, respectively clover, clover, French bean and chickpea. Media and growth conditions for *Rhizobium*, and the pea inoculation tests have been described¹⁵ as has the method of doing bacterial crosses¹³. The crosses were plated on minimal medium and where appropriate, rifampicin ($20 \mu\text{g ml}^{-1}$) to counterselect the donor plus kanamycin ($20 \mu\text{g ml}^{-1}$) to select transconjugants. Colonies were purified by streaking twice on the same selective medium and cultures were grown and used to inoculate two peas (var. Wisconsin Perfection). The plants were monitored for the appearance of nodules. After 3–4 weeks the plants were scored for nodulation and the ability to reduce acetylene. Nodules from each of the peas nodulated by the transconjugants were excised, surface sterilised, crushed and the contents plated on complete medium. About 50 single colonies were replicated to appropriate selective media. In all cases the colonies had the appropriate markers.

was not a high-frequency donor of chromosomal markers in the cross between T3 and strain 6015 as, when selection was made for the transfer of *phe1*⁺ or of *trp12*⁺, progeny arose at frequencies of only $\sim 10^{-8}$. Thus nodulation ability was transferred at frequencies $\sim 10^6$ -fold greater than these alleles.

Earlier studies showed that the plasmid R68.45 mediated chromosomal recombination between *R. leguminosarum*, *R. trifolii* and *R. phaseoli*¹². The strains used were derived from the same parents as were strains 6015, 6661 and 1233 respectively. These studies have since been extended (A.W.B.J., unpublished observations). In separate crosses 16 different alleles were transferred from *R. trifolii* to *R. leguminosarum*. From each cross 20–30 recombinants were tested for their ability to nodulate clover but this was not co-inherited with any of the alleles tested. The location of the alleles chosen on the *R. leguminosarum* chromosome¹³ was such that if any gene(s) for host specificity was chromosomally located and was transferred and expressed then we would have expected to have been able to map it. This negative result provided indirect evidence for plasmid involvement in host specificity.

Higashi⁵ concluded that the ability to form infection threads on clover could be transferred by way of a plasmid from *R. trifolii* to *R. phaseoli*. He did not say whether the transconjugants could still nodulate their normal host. We do not know why, in our study, most interspecific transconjugants nodulated both peas and the host of the recipient, albeit with reduced efficiency in both cases. It is possible that the introduction of the plasmid did not replace the genetic information needed by the recipient strain to nodulate its normal host. Perhaps the presence in the same cell of genetic information needed to nodulate host plants of two different cross-inoculation groups causes it to be deficient in its nodulation of either. The single *R. phaseoli* transconjugant that nodulated peas well but beans barely at all may represent a case where the information needed to nodulate beans had been lost.

Physical isolation and characterisation of the plasmid(s) involved may help to clarify their role in symbiosis. Such analysis may be complex, as *R. leguminosarum* strain 300, the parent of the strains used in this study, contains three large (MW > 10⁸) plasmids¹⁶.

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Dynamics of protein hydration by quasi-elastic neutron scattering

THE importance of water and its properties for biological systems makes the analysis of the dynamics of protein hydration of special interest^{1–3}. It has long been recognised² that neutron scattering can, in principle, provide the most complete description of molecular motions in hydrated biopolymers. Here we report the first results of an application of high-resolution quasi-elastic neutron scattering^{4–7} to the dynamics of protein hydration. A neutron beam incident on a biological sample produces scattered radiation that consists of a coherent part (that is, one that gives rise to an interference pattern which, in its Bragg scattering, contains the structural information^{8–10}) and an incoherent part that cannot give interference effects but exhibits spectral changes which carry information on dynamic processes. Quasi-elastic neutron scattering explores the low-frequency region of the spectrum characterised by energy transfers of the order of, and less than, 1 cm^{−1}. Because of the very large incoherent scattering cross-section of protons (80 b) relative to all other nuclei (<0.4 b) present in natural biological samples, the incoherent scattering is almost entirely due to hydrogenous groups and water molecules. By replacing some or all of the carbon-bound hydrogens by deuterium (2 b), dynamic contrast factors of up to 15 can be attained so that a wide range of contrast experiments becomes possible.

In the case of hydrated biopolymers, there are three main classes of motions that may be investigated by incoherent neutron scattering: polypeptide side-chain motions¹¹, intramolecular modes involving subunits and larger segments of a macromolecule, and motions associated with the water of hydration. Recent theoretical work on protein dynamics has considered thermodynamic fluctuations¹², the fluid-like nature of thermal motions on a picosecond time scale¹³, segmental flexibility¹⁴ and folding¹⁵, and computer simulations of the solvent structure¹⁶. In an effort to exploit the potential of recent advances in high-resolution neutron spectroscopy^{6,17,18} in studies of this kind, we have examined the dynamics of water molecules interacting with a fully deuterated globular protein at low to moderate levels of hydration.

We used two biosynthetically fully deuterated samples of the multimeric chromoprotein C-phycocyanin (MW 190,000) extracted from *Synechococcus lividus*^{19–21}. Blue-green algae contain large amounts of phycocyanin and are capable of synthesising it in extreme environmental conditions. Current interest in protio- and deuterio-phycocyanin relates primarily to its photochemical properties and to the opportunities it offers as a model system for studying the inter- and intramolecular forces involved in folding and aggregation processes. One sample consisted of stacks of protein films (surface density ≈ 5 mg cm^{−2}) cast on thin Al foils, the other was in the form of fluffy aggregates of wisp-like particles obtained by drying from aqueous solutions. The IN10 backscattering spectrometer¹⁷ at the Institut Laue-Langevin, Grenoble, was used to record quasi-elastic spectra at 5 or 6 scattering angles between $2\theta = 9.8^\circ$ and 110° (corresponding to momentum transfers $k = (4\pi/\lambda_0) \sin \theta = 0.17$ and $1.64 \text{ \AA}^{-1}$) within a spectral window of 0.07 or 0.11 cm^{-1} centred on the incident neutron wavelength $\lambda_0 = 6.27 \text{ \AA}$ (Fig. 1). To obtain reliable and consistent values for the small quasi-elastic broadenings, ΔE , expected for surface scattering from a deuterated biopolymer, the spectra measured for the hydrated sample were analysed, not with respect to the instrumental resolution width W_0 of the quasi-elastic line (as obtained for a purely incoherent scatterer such as vanadium), but differentially with respect to the width W_r obtained for the identical, dry or very slightly hydrated sample. Three wet-minus-dry difference spectra determined for intensity-normalised lines are shown in Fig. 2. For the same reasons, this line-shape analysis was not

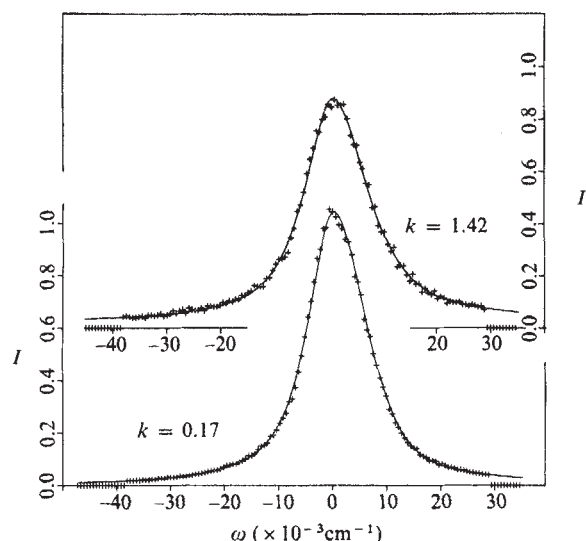


Fig. 1 Examples of quasi-elastic spectra measured (+) for phycocyanin film stacks hydrated at relative humidity $RH \approx 90\%$ H_2O (temperature $24^\circ C$). I = neutron counts corrected for detector efficiency (arbitrary units); ω = energy transfer. Momentum transfer k ($\text{\AA}^{-1}$) corresponds to scattering angles $2\theta = 9.8^\circ$ and 90° ; resolution widths W_r (dry sample) were 0.0129 and 0.0143 cm^{-1} , respectively. Solid lines are analytical fits using asymmetric Lorentzian (PP) profiles²². The spectra shown were obtained by a backscattering technique¹⁷ as follows. Cold neutrons from the liquid deuterium moderator (20 K) in the reactor are collimated along a 55-m guide tube. This beam consists of neutrons with a wide distribution of wavelengths in the $2\text{--}12 \text{ \AA}$ region. Reflection from a silicon monochromator crystal produces a highly monochromatic line of $\lambda_0 = 6.2708 \text{ \AA}$ neutrons. By imparting a periodic translational motion of a few hertz to the monochromator, the neutrons selected by it are Doppler-shifted with respect to the fixed sample and continuously scan a spectral window of $0.02 \text{ \AA}$ in wavelength or 0.11 cm^{-1} in energy centred on λ_0 . In a sample consisting of nuclei moving relative to each other, each elementary scattering event between neutron and nucleus is accompanied by small energy changes. Neutrons scattered by the sample are energy-analysed by 180° reflection from a fixed array of silicon crystals positioned at the scattering angle 2θ observed. The crystals forming this array reflect neutrons back on to a detector located very close to the sample.

carried out by means of conventional Lorentzian fitting routines. Instead, we attempted to define a less model-dependent measure of the broadening (denoted ΔE^*) based on an integral rather than a local property of the quasi-elastic line and therefore more sensitive to non-Lorentzian line-shape changes in the wings (for details, see Figs 2 and 3).

The variation of ΔE with momentum transfer k is of principal interest here. The significance of k is that the motional properties of the sample (that is, in the present case, the proton self-correlation function) are probed over a scale length of about $2\pi/k$ ($\text{\AA}$). Thus, the macroscopic limit of classical diffusion is observed as $k \rightarrow 0$, and the behaviour of $\Delta E(k)$ for $k > 1$ to $2 \text{ \AA}^{-1}$ is dominated by the short-range translational and rotational motions. The detailed form of $\Delta E(k)$ has been investigated both theoretically and experimentally for a variety of dynamic processes^{4-6,23}. The simplest case is that of continuous Fick's law diffusion for which $\Delta E = hDk^2/\pi$ where D is the self-diffusion coefficient and h is Planck's constant. The dependence of ΔE on k measured in our experiment is shown in Fig. 3. It is seen, first of all, that $\Delta E(k)$ exhibits an oscillatory structure for both the film stack and the 'fluffy' sample, with a first maximum in the broadening between $k \approx 0.3$ and $1 \text{ \AA}^{-1}$ at all H_2O hydration levels. This result clearly rules out proton diffusional motions according to Fick's law or any other bulk diffusion process for which the linewidth would increase monotonically with k and then level out at the highest k observed. Also, the effective

diffusion coefficients obtained from Fig. 3 as $k^2 \rightarrow 0$ fall in the range of 10^{-7} – $10^{-8} \text{ cm}^2 \text{ s}^{-1}$, and this is one or two orders of magnitude smaller than the coefficients of diffusion observed for water in paucimolecular layers or in bulk.

For an ensemble of incoherently scattering particles migrating on a lattice of sites, model calculations^{23,24} indicate that line-width changes similar to those seen in this experiment occur whenever the residence time τ_0 of a particle at a site is large compared with the time during which it moves to a neighbouring site. The qualitative behaviour of the line-broadening results summarised in Fig. 3, if due primarily to incoherent scattering from sorbed water molecules at the protein surface, would therefore suggest water proton motions between discrete hydration sites separated by distances of the order of $1/k_{\text{max}}$ where k_{max} is the position of the first maximum in $\Delta E(k)$. The observation of lower k_{max} values for the fluffy sample seems to be consistent with this interpretation. Oscillatory structure of the kind observed here, but with higher k_{max} values, has been widely discussed in the context of neutron scattering from protons diffusing on interstitial lattice sites in metal hydrides^{6,23}. Although the migration of water molecules on or near the surface of a protein cannot be expected to conform to any kind of regular lattice of hydration sites, we have nevertheless attempted to fit Chudley–Elliott (CE) jump-diffusion models²⁴ to the data. Because of the very limited number of data points for $\Delta E(k)$ obtained so far, the curves shown in Fig. 3 and the numbers derived from them must be regarded as illustrative of the technique rather than definitive.

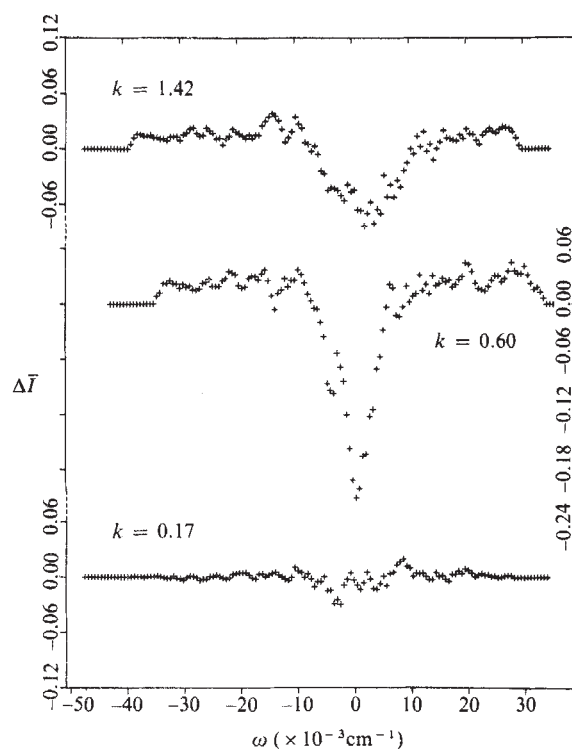


Fig. 2 Wet-minus-dry difference spectra (sample and hydration conditions as in Fig. 1) for momentum transfers k ($\text{\AA}^{-1}$) corresponding to $2\theta = 9.8^\circ$, 35° , 90° . For each scattering angle 2θ , the PP-fit to the resolution line $I_r(\omega)$ (dry sample) was peak-normalised such that $I_r(0) = I_{r0} = 1$, then integrated within $\omega = \pm\infty$ to give a reference intensity A_r . The PP-fit to the broadened line $I_s(\omega)$ (wet sample) was integrated and intensity-normalised according to $A_s = A_r$, to give a peak intensity $I_{s0} < 1$. The normalisation factors thus determined were applied to the measured spectra before subtraction. The bottom trace, representing the difference of the essentially unbroadened lines at the lowest scattering angle analysed, demonstrates that overall line shape changes with r.m.s. deviations of less than 2% are observable.

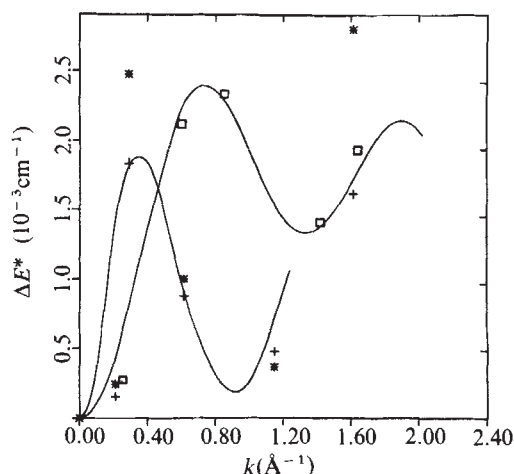


Fig. 3 Quasi-elastic broadenings ΔE^* as a function of momentum transfer k for film stack sample ($\square$) (hydration conditions as in Fig. 1), 'fluffy' sample at low (+) ($RH = 10-30\%$) and intermediate (*) ($RH = 30-50\%$) H_2O hydration levels. Resolution lines $I_r(\omega)$ (dry sample) were peak-normalised as in Fig. 2; broadened lines $I_s(\omega)$ (wet sample) were peak-normalised in the same way so that now also $I_s(0) = I_{s0} = 1$ but $A_s > A_r$. Then $\Delta E^* = A_s - A_r$, and for purely Lorentzian profiles, $\Delta E^* = \pi/2(\Delta E_{Lorentz})$. Comparison with Lorentzian-fitted profiles gave values of 1.8–1.9 for the proportionality factor instead of $\pi/2$, demonstrating the presence of non-Lorentzian line shape components.

Except at very low k , the film-stack broadenings agree reasonably well with an isotropic jump model for which $\Delta E \sim 1 - (\sin ka/ka)$, the jump distance $a = 5.5-6 \text{ \AA}$ and the residence time $\tau_0 = 5-10 \text{ ns}$. The broadenings measured for the fluffy sample, on the other hand, exhibit a pronounced first minimum at $k = 0.8-0.9 \text{ \AA}^{-1}$ that cannot be modelled in any simple way. The lower curve in Fig. 3 represents a weighted superposition of $\Delta E(k)$ resulting from one- and two-dimensional CE models, the reason for this being that at lower hydration levels one might expect the migration of water molecules to proceed preferentially along polypeptide chains at the surface. Here, $a = 7-9 \text{ \AA}$ and $\tau_0 = 15-30 \text{ ns}$. This sample was hydrated continuously from the dry state at a very low rate; the hydration number at the end of the experiment, estimated from the increase in total neutron counts, was $300 \pm 50 \text{ H}_2\text{O}$ per subunit. The number N_r of amino acid residues offering primary hydration sites is difficult to assess because the surface structure and the degree of aggregation of subunits are not known in sufficient detail. Rough estimates would place this number in the range $100 < N_r < 200$ per subunit. The distances d_r corresponding to this range may be evaluated by covering the surface with a hexagonal grid and regarding each vertex point as a potential hydration site. This gives $d_r = 80 \times N_r^{-1/2} \text{ \AA}$ or $7.9 > d_r > 5.6 \text{ \AA}$, that is, a range of values that agrees well with the jump distances deduced from the broadening data by means of simple CE models.

In conclusion, we have presented the first high-resolution neutron spectra showing the interaction of water molecules with a fully covalently deuterated protein. We have observed a hydration-dependent maximum in the quasi-elastic broadening which, at sub-monolayer coverages, seems to be due to the jump-like migration of water molecules closely associated with or bound to the surface of protein molecules. Although more data need to be collected to substantiate this interpretation, as well as theoretical efforts to develop the analysis of quasi-elastic neutron scattering from biopolymers, the results given here demonstrate clearly that details of protein hydration processes down to energy changes of the order of $10^{-5} k_B T$ or 10^{-3} cm^{-1} are now within reach of neutron spectroscopic techniques. A very exciting prospect of the contrast variation method is the possibility of experiments aimed at distinguishing dynamically between the water of hydration and intramolecular fluctuations.

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Erratum

In the article 'Model of the fine-grain component of martian soil based on Viking lander data' by Nussinov *et al.* *Nature* **274**, 859, lines 1 and 2 on page 861 should read: 'can be suspended in the atmosphere, the fact that the mean diameter which can be obtained on this basis is $1-2 \mu\text{m}$ '. In line 11 on page 861, for 10^4 mol g^{-1} read 10^4 nmol g^{-1} .

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CHRISTMAS BOOKS

Aesthetics and scientific progress

Martin Pollock

TWO THOUSAND years ago the distinction we now make between 'Art' and 'Science' would have been meaningless. The so-called 'Two Cultures' would both have been incorporated into 'Ars' and 'τεχνη'. For the past 350 years or so Art and Science have been more sharply differentiated than ever, the latter having developed at an ever-increasing speed to alter our whole interpretation of the Universe and provide technological advances and practical solutions to a large number of our problems (if not to the really vital ones such as how to get on with each other). The edifice of Science is magnificent; yet, somewhat ironically, it has been built up at the expense of increasing restriction in the philosophical concepts on which it rests. "We forget", wrote Whitehead in 1926, "how strained and paradoxical is the view which modern Science imposes on our thoughts". Most of us do not just "forget"; the possibility never even occurs to us. We are blinded by the brilliance of our understanding and the triumphs of our technological achievements.

Nevertheless, within the past few decades, there have been increasing efforts by philosophers, artists and scientists themselves to challenge the assumptions on which Science is traditionally thought to be based and to explore the extent to which the principles and practices of Art and Science respectively may contribute to each other and be seen to share common factors in the forces which drive human beings to express themselves in these two rather different ways. After all, most people are now prepared to agree that there can be beauty, understanding and creative elements in both. In particular, scientists have to extricate themselves from the vicious circle which they themselves create when "what we notice is selected by our concerns and our concerns are excited by what we notice", as Geoffrey Vickers points out in Judith Wechsler's book, *On Aesthetics in Science* (MIT Press: Cambridge, Massachusetts, and London, \$12.50; £8.75). The same point, incidentally, was made by Blake 200 years ago when he remarked that "if it were not for the poetic or prophetic character, the philosophic and the experimental would be at the ratio of all things and stand still, unable to do other than repeat the same dull round over again".

To attempt a re-analysis (albeit limited) of Science at a time when its functions in

human society and the implications of its achievements are being questioned, is all to the good. Judith Wechsler's contribution has been to edit a collection of six essays by scientists all of whom "maintain that Aesthetics is a crucial factor in the scientific process". She quotes Poincaré in her introduction as saying that "in mathematics, the useful combinations are the most beautiful" and that "it is more important to have beauty in one's equations than to have them fit experiment . . ." (!) (which incidentally calls to mind Watson's and Crick's comment on their structure for DNA being "so pretty that it must be true").

The first two articles in *On Aesthetics in Science* concern the parallels between structural patterns in Art and in Nature. Cyril Smith emphasises the repetitive build-up of modular units to form a spatial hierarchy of recognisable forms in pictorial art and natural systems in physics, chemistry and biology. His description of aesthetic pleasure is "what is left over when what is expected has been cancelled out", which is charming if rather personal. It is, however, surprisingly similar to what Philip Morrison says, in the second article (on "Broken Symmetries") when he maintains that highly satisfying works of art are characterised by "faults in an otherwise regular pattern". Both these essays, stressing the analogy in the significance of similar spatial patterns in Art and Science, are open to the objection that the mind is a unity; it is not artificially divided into an Arts section and a Science section. Some congruency in pattern interpretation is to be expected. The main question is to what extent do these parallels penetrate into the deep structures of the two 'disciplines' so as to have some real significance. This needs much further research before it can begin to be properly tackled.

Arthur Miller gives an interesting (though rather difficult) analysis of the controversies in modern sub-atomic physics in terms of the distress engendered in some physicists by having to face pressures to abandon visualisation in their theorising. It is argued that the wave theory of radiation developed by Schrödinger (and supported eventually, with relief, by Bohr) was inspired by his need to visualise the elements involved; it was opposed by Heisenberg, who preferred non-visual thinking and who thought that Schrödinger's approach was "disgusting". Analogous aesthetically orientated influences operated, so Miller maintains, in the

quantum/continuum "antithesis".

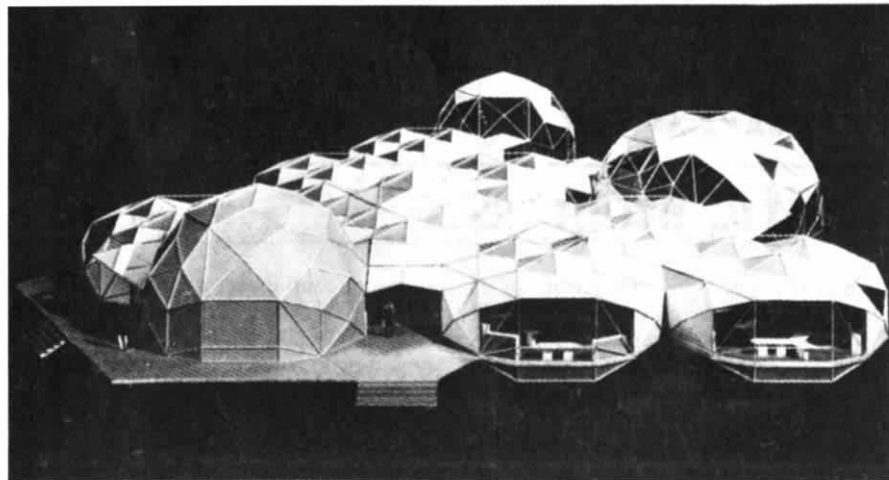
Seymour Papert extends Poincaré's theme that "Aesthetics" (working unconsciously) rather than logic "is the distinguishing feature of the mathematical mind", by analysing sequences in mathematical proofs on the basis of the aesthetic pleasure they can provide ("aesthetic guidance"). It is unfortunate, however, that the principle theorem so presented, being an entrancingly beautiful proof that $\sqrt{2}$ is an irrational number, is desperately confused (at least for the general reader) by a series of shattering misprints on pp112 and 114.

Howard Gruber illustrates what he calls "aesthetic moods" by concentrating on Darwin's evolutionary "trees" and other images used for developing scientific theories. It is the image of a concept that we love, he stresses, not the concept itself. And he rightly points out that there is a beauty in certain types of complexity as well as in simplicity. Indeed, theories do not have to be simple, as some think, in order to be "true" (that is, acceptable); they become simple with familiarity and by the need for simple presentation if they are believed to be true and have to be expounded.

Finally, Geoffrey Vickers develops forcefully and convincingly what is surely one of the most important points of all: namely, that there are indeed two different modes of "knowing" available to the human mind (two "cultures" one might say) based respectively on rationality and intuition, but these do not correspond rigidly, or even approximately, to what we call Science and Art.

The conclusion from this collection of essays is that some fundamental re-definitions and re-thinking are necessary in our approach to Art and Science. Although the pictures presented of the relationship between Aesthetics and Science are inevitably tentative and blurred, the objectives behind the book are excellent. There is a well compiled general bibliography. The articles themselves are provocatively stimulating and, although sometimes inclined to be rather turgid and verbose, are well worth studying by all those not already hopelessly rigidified in their beliefs of what Science is about. We should be grateful to Judith Wechsler for a courageous attempt to freshen and broaden our attitude.

Peter Pearce's well produced and well illustrated book on structural design, *Structure in Nature is a Strategy for*

Taken from *Structure in Nature is a Strategy for Design*

schools and factories. He has built many models, though, so far, no full-scale constructions. His buildings bristle with angles and slopes in all directions. They look fascinating and some (though certainly not all) are rather beautiful. Most appear bizarre to the conventional eye. In spite of the theoretical diversity available, one might suppose, from all the permutations and combinations possible of standard unit building elements provided (nodes for a variety of fixed angles, triangular plates of different sizes and shapes with opaque or transparent panels, and so on), the completed structures do not appear as different from each other as one might expect—probably because our orthogonally prejudiced eyes are blinded by all those angles. But they may indeed represent at least one important trend in the architecture of the future. Some of the constructional principles are surely excellent; so indeed is the attitude towards economy of cost and effort. But the basic Aesthetics behind these designs is elusive probably I think because no real attempt has been made to develop or expound it—if indeed it really exists. The practical elimination of the monumental curve seems to impoverish many of the designs and the suppression of the right-angle introduces a sensation of insecurity. But such reactions are so often based on personal and fashionable prejudice. To many of us, Pearce's ideas are unfamiliar, exciting and original. They surely deserve further exploration at a practical level. □

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Design (MIT Press: Cambridge, Massachusetts, and London, \$45; £31.50) with its curious and rather misleading title, is in one sense a formal geometrical analysis of the hierarchy of natural and man-made structures considered by Smith and Morrison in Wechsler's book. But it is really only tangentially relevant to the principles of Aesthetics as applied to Science and technology. For the general reader it is often rather heavy going. It is an exhaustive (and at times somewhat exhausting) compendium of different types of space-filling polyhedra based on a limited number of simple different polygonal surface structures, pieced together in a variety of ways. The objectives in design, both in natural and man-made structures, are—and must be—economy of energy, effort and cost (closest packing of a minimal number of different types of constructional 'unit') combined with optimal efficiency (strength for resisting internal and external strains) and maximal diversity (to meet a wide range of different environmental requirements). This leads to numerous structural similarities between natural patterns (snow-flakes, soap bubbles, honeycombs, pineapple skin, cracked mud, giraffe skin markings) and man-made structures (for example, the famous Buckmaster Fuller 'geodesic dome') according to the exigencies of different situations.

One of the more significant points emphasised by Pearce is the superiority in strength and stability of the triangle over the square as a unit side for complex polyhedra subject to various strains and stresses. This results in a series of three-dimensional structures in which the right-angle occurs relatively rarely and walls and ceilings, as we know them, have almost disappeared. The box-like construction of most contemporary architecture is thus largely abandoned. Nor are there any 'real' curves—except in so far as the so-called 'saddle polyhedra' are involved, these having sides of minimal surface area formed when the unit polygon is skew (that is, non-planar). With special properties of strength and stability, they are involved in the construction of tunnels,

continuous surfaces and solidly enclosed labyrinths.

A huge range of different polyhedra is described systematically in terms of their constituent elements, illustrated by a vast number of diagrams and photographs, which will make most ordinary readers dizzy trying to work out what a monster composed, for instance, of hemi-saddle digonal disphenoids interdigitating with octahedrally truncated rhombic dodecahedra would actually be like. Yet this could, no doubt, be of great value to the experimentally minded architect really trying to break new ground in functionally improved design and cheaper construction costs.

Pearce is very production-conscious and has his own company, *Synestructures*, for developing his ideas which range from children's playground toys to homes,

Metaphor of the mechanical body

The Body in Question. By Jonathan Miller. Pp. 352. (Jonathan Cape: London, 1978.) £7.95.

THE medieval commentator and teacher, faced with a previously unknown book and attempting to understand it himself and explain it to others, asked of it a formal rote of questions designed to relate it to the known intellectual world. Sometimes the modern reviewer is in the same position when attempting to relate the book in question to the particular interests and expectations of the readers of the journal for which he is writing.

To explain the *Body in Question* our scholastic predecessors would undoubtedly have thought it necessary to bring into play the whole apparatus of Aristotelian causality which, given ingenuity, could explain anything: the efficient cause of the book is Jonathan Miller, intelligent and charming physician, erstwhile historian of medicine, and arch-communicator; the final cause of the book is the opportunity to take advantage of the advertising furnished by a related

13-part television series; and the material cause is some two and a half pounds of glossy paper. The remaining cause is the formal: the blueprint in the author's mind, translated into the format and content of the book.

The author at once tells us what the book is not: neither a history of medicine nor a complete survey of physiology, and it remains the reviewer's job to tell the reader what the book is. It is an attempt to use historical and modern evidence to present physiology in terms of physical science. To put it another way, it is pop physiology with history to tell us how the metaphor of the body as a machine came true—"the story of the identification of the machine within the ghost". This is a serious argument and it deserves serious review. Let us look at the history.

Perhaps the best feature of the book is the discussion of the limits of perception: how the brain's past experience leads it to impose a pattern on the data of perception, enables it to choose between alternative perceptual interpretations, and how it distorts perception by being "theory laden". The author shows clearly that this process has in the past led to "false" scientific ideas, and this is

a hopeful start to the historical problem of reductionism or mechanism. But the brain distorts historical as well as scientific knowledge, and although it may be tempting for the physician to argue that "[Modern] Natural science is the native idiom of the brain made explicit", such a statement leads to a historically suspect endorsement of Miller's whiggish main thesis, that the true, mechanical, account of the body emerged only in the last century. It is not true that the medicine of the Middle Ages was characterised by "monkish superstitions", and it is not true that eighteenth century medicine was a "tissue of contradictions" with "no consistent intellectual standards and no organised body of scientific principles", or that its practitioners were "unable to make sense" of the doctrine of irritability. Miller holds that at last by the 1830s "the medical profession was almost within reach of coherent scientific credibility", but in fact it was precisely credibility of this sort that made Riola oppose Harvey, and the College of Physicians of London oppose reform, two centuries before.

To support his argument about the development of the idea of the mechanical body, Miller naturally looks at the historical use of mechanical analogy (not metaphor) by medical writers, particularly in relation to involuntary motion and the beat of the heart. This again is a serious historical argument and the influence of the mechanical pump on Harvey's ideas has been seriously discussed by historians: it was probably negligible in *De Motu Cordis*, but it is much more likely that Harvey was influenced by the notion of a valve as illustrated by the analogy of sluice gates. This kind of argument needs very close examination of the sources, and Miller's examination of Harvey and the circulation is unsatisfactory.

Fifteen hundred years before, Galen had used the analogy of a suction-pump, and Erasistratus, an ancient even to Galen, thought the heart was a force pump, as Harvey well knew. The descriptions and relationships of Galen's physiological system, Harvey's, and mechanical analogy, are full of errors. Apart from technical mistakes, to call Galen's system "an industrial plant halfway between a brewery and a blast furnace" is to grossly exaggerate Galen's use of mechanical analogy, and to say that Harvey's quantitative arguments mark him as the "first modern biologist", who took "an altogether mechanical view of the function of the heart" and who wrote a book "with the force of great literature" is to be blinkered by twentieth-century hindsight to Harvey's great and constructive conservatism.

The author's argument is further supported by a great number of illustrations often so striking as to rival the text

for the reader's attention. A dramatic, if hardly beautiful, double spread of a new born infant illustrates only that "The possession of body may be a necessary condition of being a person but it is not a sufficient one"; an illustration of a pot being turned has the solemn caption "Pottery is a typical way of making things happen . . ." and supports only a description of the role, or lack of a role of the hands in faith-healing. Is not a photograph of a large man chasing a naked boy down a dark corridor a gratuitous illustration of "A nervous system which can recognise and react to threats as and when they occur"? The

technique is pure television, frozen on to the glossy page, and the reader emerges from the book like an after-dinner viewer switching off a cultural documentary with a faint feeling that his mind has been improved, but unable to quite remember the intellectual meat in the very professional presentation. No biologist will be surprised by the ideas in the book, but the layman will find that it does tell him a large number of interesting things about his own body.

R. K. French

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Tree appreciation

IT is not surprising that the ravages of Dutch elm disease should have stimulated an attempt to produce a compilation properly celebrating the role of elms in our landscape and culture. The result of such an attempt is Gerald Wilkinson's much promoted *Epitaph for the Elm* (Hutchinson: London, £6.95). It begins with chapters on the elm in poetry and painting; but whereas the poets—particularly Clare, Edward Thomas and Betjeman—are well represented, the painters have not fared so well. Constable is featured prominently with some fine illustrations—but also some poor ones. One whole page is devoted to a postcard size view of Dedham Vale in which hardly any trees can be seen. More recent landscape painters such as John and Paul Nash have been undeservedly ignored.

The next section deals with the botany of the elm, and forty pages (a quarter of the book) are devoted to the genus *Ulmus*. Unfortunately Mr Wilkinson proves to be an inadequate guide through the labyrinth of elm taxonomy. The most widely held view today is that the Wych elm (*U. glabra*) is our only native elm,

and that in the pre-Roman period there was a series of introductions to Britain of various clones of the European field elm (*U. carpinifolia*) and of *U. carpinifolia* × *U. glabra* hybrids. Other hybrids are thought to have subsequently been formed in this country. This view, based on the meticulous and scholarly research of R. H. Richens, receives no attention from Mr Wilkinson. Rather, he prefers to describe in great detail the older work of R. Melville in which a number of elm types were described as distinct native species, and he waxes very indignant that the recent editions of Clapham, Tutin and Warburg do not accept them. One's doubts as to whether Mr Wilkinson could recognise some of these elm 'species' himself are reinforced when, in dealing with the Dutch elm, that distinctive clone of southern England, he states "I have not been able to satisfy myself that [this elm] exists as a recognisable type in the English countryside".

His confusing treatment of our elm population leads him to make some very questionable statements in the next chapter, that on the elm in prehistoric Britain. It is followed however by a much better chapter on the historical period, with quotations from John Aubrey, John Evelyn and others, and by nicely illustrated sections on the past and present uses of elm wood.

Sadly, however, the book ends with a very weak section on Dutch elm disease. It is clear the Mr Wilkinson has a very imperfect understanding of the biology of the disease, and this leads him to make wild remarks such as, "even though elm bark beetles are not supposed to lay their eggs in shoots of less than ten years of age some of the pollarded elms in Suffolk are dead".

In summary, despite the fact that it is attractively presented and contains some good photographs, *Epitaph for the Elm* is too flawed to be an adequate tribute.

The Dutch elm disease epidemic is undoubtedly at least partly responsible for the current interest in trees of all kinds, and this year two new guide books have appeared. Roger Phillips' *Trees in Britain, Europe and North America* (Pan: London, paperback £3.95) is a photographic guide



English elm

Photo: Forestry Commission

to "500 of the most common and important trees of the temperate climatic region". It is aimed at people with no botanical training and consists of two parts: the first comprising fifty pages of leaf photographs from which the initial identification is made; the second consisting of brief descriptions of the trees in alphabetical order, illustrated with photographs of the flowers and fruit. The "leaf index" should prove quite useful, although some of the photographs are blurred and most of the names on the first page have been transposed. The photographs of the flowers and fruit are very attractive, although here again there are mistakes which indicate that the final stages of publication were over-hurried. Nevertheless this is a book which many will find helpful and enjoyable.

The late Herbert Edlin's *The Tree Key* (Warne: London, hardback £5.95; paperback £3.95) relies on paintings rather than photographs, and there are pleasing pictures showing tree shape, summer and autumn foliage, leaves, flowers, fruit and seedlings. The main problem is that the book has the formidable ambition of being "a really practical guide for those seeking trees on either side of the Atlantic". As it is pocket-size this means that, for most trees, identification can only be to the generic level. Oddly, in view of the name, there is no key in the sense that one can systematically track down any particular tree. There is a series of classifications (leaves on the basis of shape, soft fruit on the basis of colour) but they are incomplete. Thus one cannot find *Catalpa* listed among the genera possessing "fruits with many seeds".

No doubt there are financial attractions in producing a book that can be sold on both sides of the Atlantic, but I wonder if this is really a sound policy, particularly with such a small book as *The Tree Key*. It means that some of the very limited space is devoted to species such as the Rock elm (*U. thomasi*), that are almost non-existent in Europe.

Trees by Andreas Feininger (Penguin: Harmondsworth, UK, paperback £3.95) is described by its author as "a tree appreciation book, rather than a guide or picture book". Sample chapter headings indicate its approach: "The oldest living things", "Leaves", and "Fall colours". It is illustrated by 160 photographs, mostly taken in North America but also some in Europe. Many of these are beautiful and evocative, in particular those of Californian trees such as the Redwoods and the Bristle-cone pine. There are also some pleasing black-and-white pictures of bark and weathered wood. The accompanying text of some 100 pages is lucid if slightly repetitive.

Mr Feininger's great interest in the conservation of the North American forests comes through strongly, and the book ends with a section—not very appropriate for British readers—on con-

servation societies in the USA. The text seems to be unchanged from the original American edition of 1968 so that some of the facts, figures and attitudes are a little dated. For example, he states that in the USA consumption of wood for fuel has declined steadily through the

past century, whereas the past-five years must have seen a marked increase in its use for this purpose. **John N. Gibbs**

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Nature's traces

PICTURES add a vital dimension to books on natural history and, with the improvement in techniques for printing illustrative material, more and more reasonably-priced illustrated biology books are coming on to the market.

Nature Detective by Hugh Falkus (Gollancz: London, £6.95) is a most handsomely produced book with many excellent black-and-white photographs. Many of these are by Niko Tinbergen, whose knowledge and enthusiasm have obviously been a major source of inspiration to the author. The pictures are the most important feature of the book and illustrate very clearly the insights that can be gained into the behaviour and ecology of animals by careful examination of the signs, tracks and droppings that they leave behind. The text provides some background information about the animals and localities illustrated.

David Stephen's *Guide to Watching Wildlife* (Collins: London, £1.75) is a paperback which gives advice on where, when and how to watch birds and mammals by day and by night. It is richly illustrated with the author's own drawings and photographs, the latter testifying to Mr Stephen's skill in practising what he preaches. The emphasis of the book is on practical skills, such as how to watch badgers, and it provides a very useful guide for those who wish to explore nature more actively.

Recording Natural History Sounds by Richard Margoschis (Print and Press Services: Barnet, London, UK) is a very specialised paperback book suitable for a handful of enthusiasts and for researchers working on animal sounds. It contains much detail on principles and techniques and a number of photographs which are not generally very informative, many of them simply showing various types of tape recorder.

Nature Day and Night by Richard Adams (Kestrel: London, £3.95) is an attractively produced book, but one whose purpose is not very clear. Its theme is the way in which animals and plants are adapted to the alternation of day and night. The book has two rather different components. Richard Adams contributes a series of essays on, for example, "the meadow by day"; these tend to be rather whimsical in style. Max Hooper has written a series of scientific essays on specific subjects such as biological clocks, and David Goddard provides attractive

illustrations, those for the general essays being double-page spreads crammed with animals and plants, sadly many of them too small for accurate identification. The scientific sections are excellently illustrated with drawings and diagrams and are the more successful aspect of this book.

Exploring Nature by David Hall (Hamlyn: Feltham, UK, 50p) is a practical guide for children and seeks to encourage active investigation and experimentation. Projects discussed include setting up aquaria, trapping soil animals, making spore prints from fungi and investigating photosynthesis. There is a wealth of ideas which should not only stimulate enquiring youngsters but will also be a useful stimulus to their parents and teachers.

Another children's book, *The Young Naturalist's Guide to Conservation* by Neil Arnold (Ward Lock: London, £2.95), has the admirable aim of providing practical suggestions for young people wishing to protect and encourage wildlife in their immediate surroundings. It describes how to maintain ponds, plant trees and observe birds and other wildlife. The text is concise and clear but the illustrations are somewhat variable in quality.

The *Chatto Nature Guides* (Chatto and Windus: London, hardback £4.50; paperback £1.95) are translated from German books and are illustrated by colour photographs. Each species described is covered by a photograph and a brief text giving details of characteristics, habitat and distribution. Individual titles are *Birds*, *Wild Flowers*, *Mushrooms and Fungi*, *Insects*, *Aquarium Fishes* and *Minerals and Rocks*. The photographs are generally excellent, though in some instances are not suitable for the purposes of identification in the field. For example, the Cuckoo is illustrated by a photograph of a fledgling which lacks the characteristic long tail of the adult. At such a low price one could not expect these little books to be comprehensive in their coverage, but there are some very serious omissions. For example, in *Birds* there is no Mistle Thrush, Lesser Black-backed Gull, Kittiwake, Dunlin or Shelduck. Perhaps these deficiencies reflect the German origin of these books; the volume on birds is notably short on coastal species. A good feature of a number of these guides is that they draw attention to instances where species may be confused with one another and emphasise the points of difference. *Mushrooms and Fungi* includes a clear code to indicate which species are palatable and which are poisonous. *Aquarium Fishes* includes hints on how each species

should be kept in the aquarium.

The *Penguin Nature Guides* (Penguin: Harmondsworth, UK, hardback, £1.95) have also been translated into English, from either Danish or Swedish. *Fishes of the British and Northern European Seas* has excellent illustrations by Bente Nyström to aid identification of species. There is also much interesting background information on fish and fisheries illustrated by diagrams and photographs. *Birds of Wood, Park and Garden* and *Birds of Sea and Coast* conform to the now familiar fieldguide format with excellent paintings by Lars Jonsson in which the birds look very much alive, a brief text on their natural history and characteristics, and distribution maps. *Plant Communities* incorporates the interesting idea of arranging plant species in the communities to which they belong. Separate sections deal with meadows, the

sea shore, oak forest, and so on. A problem with this book is that (as is made clear in the book) many of the species shown do not occur in Britain. This is a problem inherent in books translated from another language. Also this book shows very few species and is thus of limited use as a field guide. Finally, *Fungi of Northern Europe, 1: Larger Fungi* and *Fungi of Northern Europe, 2: Gill Fungi* have excellent illustrations, among the virtues of which is that they show much of the variety of form which each species can achieve. These attractively produced books can be recommended as a reasonably priced, exceptionally well illustrated and informative series.

T. R. Halliday

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Solving the problem of bird identity

THE world shrinks for the bird-watcher—and others—as 'special interest' package tours provide inexpensive ways to view new continents, where we discover birds of new families and orders of which perhaps we had hardly imagined the existence; and where we can often see familiar species in strange and exotic habitats. The television too takes its viewers to wild lands and curious creatures. Whatever way we travel, even if it is only by reading travel books, we need a source to tell us something of the characteristics and relationships of some beautiful bird seen hazily in the hot desert or all too briefly in the rain forest.

There are no fully comprehensive books on birds of the world other than checklists, and Dr Colin Harrison's *Bird Families of the World* (Phaidon: Oxford, £9.95) is probably the nearest that we can find at this price to such a survey of the families of birds existing in the world today (together with some extinct ones). Each family account is written and signed by one of 43 specialists and describes the numbers of species and genera in each family as well as noting the main features of their distribution, feeding behaviour, and nesting habits. About 900 species are illustrated by useful and accurate coloured drawings by Ad Cameron. Dr Harrison had used J. L. Peters' order for arranging his material but reminds the reader that there is another possibility and points out the alternative names and orders where they could be applied. The book is a 'popular' but serious and well-illustrated attempt to convey a picture of the main features of the birds of the world.

We don't have to travel far from home to have identification problems; moving

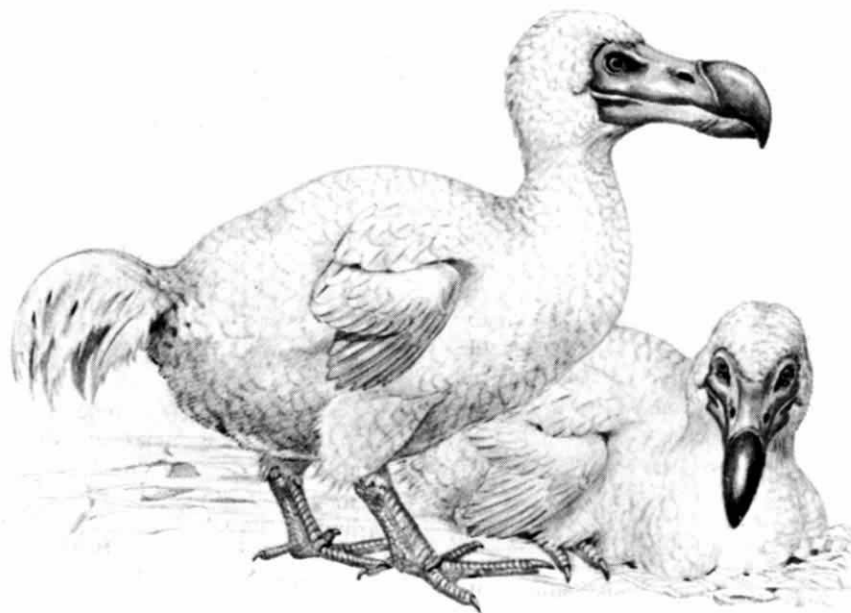
from a world range to the identification of birds in Britain John Andrews has written *Birds* which is one of the *Nature Guides* published by Hamlyn (Feltham, UK, £1.50) and designed for the many people who wish to name easily and confidently the commoner conspicuous birds they see in their gardens, in the countryside and on their holidays. Just over 200 different species are described but only 168 are illustrated by coloured photographs. Whether colour photographs are adequate for the purpose of identification is always debated; in this pocket-sized volume most photographs do their job. But the photographs of the harriers are not really clear enough and it would have been helpful to have been told the sex of the Serin and the pied wagtail—or is it a white wagtail? Also, is the bird which is labelled great black-backed gull really a great black-backed? The contrast between the grey of the mantle and the black of the primaries as well as the colour of the legs as shown in my copy would lead me to think that it

it a lesser-black-backed gull. Which all foes to show that photographs can be difficult.

John Andrews introduces his book with several paragraphs on the points to look out for when identifying birds. He gives a valuable table of habitats in which birds can be found and a short description of each species opposite each photograph. The book would make a useful Christmas present for someone, child or adult, beginning to identify birds but who does not wish to be confused by the large number of choices provided by the more advanced Field Guides.

Turning from problems of identification to other aspects of ornithology Eric Simms has written his second volume in the *New Naturalist* series, *British Thrushes* (Collins: London, £6.50), which describes some of the most familiar birds in the British Isles and in Europe. Within his compass he manages to incorporate not only the common resident thrushes, such as song and mistle thrush, blackbird and ring ouzel and the winter visitors, redwing and field fare—which also nest in very small numbers in Scotland—but also information about the thrush-like chats, stonechat, whinchat, wheatear and robin as well as these exciting vagrants which occasionally visit us from the distant north-east.

The text is illustrated by many excellent black-and-white photographs, by Robert Gillmor's pen-and-ink drawings of the displays of the different species, and by distribution maps, sonagrams, histograms and additional data supplied by experts from the British Trust for Ornithology and other organisations. Indeed the quality of the information makes this a very comprehensive volume and one which all interested in birds should have. The book, which should become a classic, is a pleasure to handle and dip into; compared with many other books produced these days it is very good value for money.



R. union solitaire (*Raphus solitarius*), a species of dodo

Guides to identify birds or their eggs are common enough and guides to bird-watching places are becoming commoner. The danger of this type of guide is that it will attract far too many people to the more delicate areas, disturb the birds and thus destroy the places they have come to see. Eric Hardy in his *Guide to the Birds of Scotland* (Constable: London, £3.95) has provided a pretty comprehensive list of places and a more comprehensive one of birds that appear in various parts of Scotland, but the bird list is rather mixed-up and makes very difficult reading. He has, however, been forbearing—careful to avoid mentioning some of the places where pressure by bird-watchers could cause big problems—and the regional maps show sufficient areas of high ornithological interest to keep bird-watchers busy for many holidays. But it is a pity that his obsessional hatred of the 'establishment' and particularly the Royal Society for the Protection of Birds, provides such a sadly sour introduction to a book which should be leading us to a greater enjoyment of birds.

While on the problems of identifying birds and their habitats, I must mention briefly another book by Dr Colin Harrison entitled *A Field Guide to the Nests, Eggs and Nestlings of North American Birds* (Collins: London, £6.50), which illustrates 622 eggs and 147 nestlings in colour. Although it is a textbook or field guide, Dr Harrison emphasises that it is for bird-lovers rather than egg collectors. For birds on the North American continent from the Arctic to the southern boundary of the USA, it incorporates basic information on habitat, nest, eggs, breeding season, incubation, nestling period, and so on. It may not appeal to the average British bird-watcher but in the United States it should become a standard work of reference.

Identification is but a first step to a deeper interest in birds and *The World of Birds* (Sampson Low: Maidenhead, UK, £3.95; the Italian author's name, Gianfranco Bologna, is rather hidden away) is the book in this collection which attempts to interest the beginner in what birds actually do. The title is rather a common one and it, or one rather like it, has been used for four or five books in recent years. What Bologna is really writing about is the way in which birds live. Many of the examples he chooses of bird biology and behaviour are common to many other recently published books on this subject and his short list of references are virtually identical to the others. What perhaps distinguishes the book is that he has used the experience of Italian authorities to supplement the standard examples. Quite clearly this book is a translation and occasionally his translator has let him down—once by referring to the "hibernation" area of the ringed plover. It is not a technical

book and it could help the bird-watcher on his way to becoming an ornithologist.

The emphasis of the last two books switches from the scientific aspects of birds to the artistic. Robert Dougall in his new book *A Celebration of Birds* (Collins: London, £5.95) has left the television scene to indulge in his retirement hobby of bird-watching. He has collected together some of his own ornithological reminiscences and his favourite ornithological verse. The chapters are centred on 15 species most of which are common enough, like the robin, blue tit and sparrows, but he also goes to the other extreme and writes about the avocet and osprey. This book does not pretend to be an ornithological treatise nor a complete collection of bird verse, but rather a book for Dougall addicts with an ornithological slant. It is illustrated by drawings, some black-and-white, and some in colour.

For a selection of 36 bird portraits painted by C. F. Tunnicliffe accompanied by a practical text written by Linda Bennett, we have the *RSPB*

Book of Garden Birds (Hamlyn: Feltham, UK, £2.95). The original paintings were commissioned by the RSPB as front covers for its old magazine *Bird Notes* or as Christmas cards, and in this book one finds a very good portfolio of Tunnicliffe paintings for a very low price. The main purpose of the book, however, is the practical text, describing the factors which make gardens attractive to birds, as well as the history of human interest in those bird gardens where bull finches might even be encouraged to take fruit. It brings to mind one of the earliest practical books on bird gardening written by the Baron von Berlepsch, which still has a lot to offer. Linda Bennett gives good advice on the art of bird gardening and of course Tunnicliffe's paintings are always a delight. It would make a very attractive Christmas present.

Peter Conder

Peter Conder edits the 'News and Comment' section of the journal British Birds, and acts as a consultant on behalf of the IUCN.

Contributions to conservation

It would be pleasant to be able to believe that the great number of people who now support nature conservation organisations in Britain and in a few other countries do so out of commitment born of knowledge. In fact, the vast majority have joined for entertainment and, perhaps, out of a vague sense of concern. Not that there is anything wrong with that but it is important that the extent and nature of their involvement is recognised. At present, the statutory and voluntary conservation bodies operate in rather favourable conditions—private affluence and leisure meaning more public interest and support, and the national economic position inhibiting some demands on the natural environment. When times really do grow hard—as it seems they must—will the support melt away just when it is most needed? Logically, the answer must be "Yes" unless in the, perhaps brief, interim every effort is made to develop people's appreciation of nature, their understanding of the complex issues that lie at the roots of conservation and, thus, their commitment.

Any book or article, broadcast or film which starts the amateur naturalist along the road of understanding how man's activities affect wildlife is contributing to conservation. *Vanishing Birds: Their Natural History and Conservation* by Tim Halliday (Sidgwick and Jackson: London, £7.50)

certainly comes into that category, treating a fairly difficult subject in a straightforward way but without avoiding the key issues.

Commencing with a general summary of avian evolution and strategies for reproduction and survival, the book examines, both in general terms and with case studies, the effect of man's past and present activities on bird populations. It is overwhelmingly clear



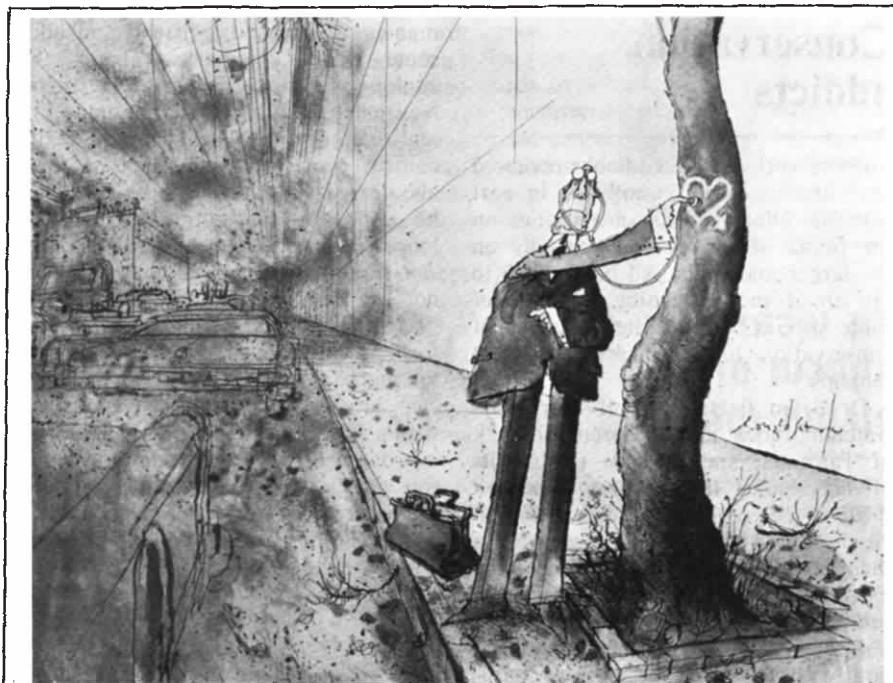
Taken from *Vanishing Birds*

that, despite a few advances, the natural environment is exploited as energetically as ever it was—often without the justification of ignorance or great need. We deplore the feckless extermination of the great auk, not only for food but as a source of feathers: to loosen their plumage the birds were dropped into vats of boiling water, heated by burning the oily corpses of their fellows. Yet we effectively ignore the fact that the only woods in which Abbot's hooby breeds are being felled in order to mine phosphates. And who protests at the stupid and criminal destruction of Amazonian forests, with their indigenous wildlife and defenceless human communities? Would there be a ban in Britain on the use of DDT if it only killed peregrine falcons and did not accumulate in human food chains also? The author argues that most of the justifications of conservation carry little weight with the general public and governments. He demonstrates weaknesses in the ethical, aesthetic, economic and scientific cases, believing that the strongest argument is ecological: we must protect the bird's environment because it is ours too. Unfortunately, many bird species could doubtless be destroyed without any significant effect on our survival or even on our wealth. The only unshakeable argument is the ethical one but its great weakness is that too few people share it.

Halliday's concluding section is a rather generalised summary of active conservation measures appropriate to endangered bird species and a wholly justified criticism of the lack of research on endangered birds. Of course, there is a dual reason for working on common species. Results are usually more certain and those results may help us to understand and solve the problems of rare and endangered birds. But if concentration on the former means neglect of the latter, the balance of research is wrong: international conservation agencies could help to redress that by careful direction of research funding.

Despite errors and omissions resulting from the fact that some information is five years out of date, which is hardly excusable in a book dealing with species whose status may be expected to change from year to year, *Vanishing Birds* is a helpful introduction to the subject, and the author's colour plates and many line drawings appear both accurate and elegant.

In *Birds of Man's World* (British Museum (Natural History)/Cornell University Press: London and Ithaca, £5.95) Derek Goodwin has taken a contrasting approach with an account of his own observations of those birds which are able to exploit man-made environments, notably the urban species



Cancer

Illustration taken from *Ronald Searle*, with an introduction by Henning Bock and an essay by Pierre Dehaye (André Deutsch: London, £14.50)—a superbly produced album of work by the graphic satirist.

which are, to most of us, the common birds of the world.

The book begins with a general summary of the ways in which man's modification of his environment have created widespread conditions which benefit a rather small number of species. Thus the deforestation of Europe, much of North America and New Zealand favours grassland species: buildings provide nesting places for cliff dwellers such as swallows, martins and rock doves which were formerly much restricted by lack of suitable sites; and the ranching of domestic livestock has permitted cattle egrets—recently confined to the Old World—to colonise America and Australia. Perhaps surprisingly, it does not discuss our one outstanding domestic example—the colonisation and spread of the little ringed plover, a species which on the Continent breeds normally on shingle bars in rivers. First recorded nesting in Britain in 1938, it has since spread widely through England to Wales and Scotland, using the growing number of gravel pits created by the expanding aggregates industry.

In some ways, this book tells one more about the author than about the birds: he is refreshingly able to find pleasure and fascination in sparrows and pigeons, fully conscious of the fact that the ordered urban world we find so reassuringly safe is to them as wild and hazardous as a rain forest is to us, and that their biology and behaviour is intrinsically every bit as worthy of study as the activity of more exotic species.

But because it is based on personal experience, the book provides an irritatingly incomplete study of the subject. A description of the urban birds of Colombo based on a day visit in 1965 leaves one very conscious of this.

A large proportion of the text discusses food sources but does not develop the subject of the impact of common birds on man's activities, nor of man's reactions.

In Britain, the inclination is still to kill birds rather than to find alternative, if more costly, solutions. Gulls, for example, exploit man's wastefulness by resorting in great numbers to rubbish tips, especially in coastal towns. When they stay to roost on the reservoirs and to breed on rooftops or in water catchment areas, man's hand is turned against them—usually by means of ill-considered and ineffectual culling programmes. In turn, kind old ladies, conservation bodies, local councils and water authorities become embroiled in acrimonious and time-wasting disputes, which inevitably divert attention from far more important land-use problems.

Derek Goodwin's final chapter looks to the "Future Imperfect". Like Tim Halliday, and no doubt most professional conservationists, he finds little cause for long term optimism.

John Andrews

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, UK.

Conservation addicts

FOUR recently published books reviewed here are concerned wholly or in part with the effect of human activities on the fauna of the world, especially on the larger mammals. All have much to say about the decreasing numbers of some species, and of the measures of conservation taken to preserve what remains.

Dr Brian Bertram spent four years watching lions in the Serengeti Park of Tanzania, and presents the results of his studies in his well illustrated *Pride of Lions* (Dent: London, £6.50). He lived for over half the time in a shed in the bush, getting to know the local lions individually and tracking some of them by putting radio transmitter collars on them after immobilisation with a knock-out shot of paralyzing drugs. His book gives a full account of the life-history of lions, enlivened by stories of his experiences and adventures. He adds chapters on the leopard and the cheetah in which he contrasts the solitary life of these species with the social life of lions. He concludes by discussing the evolution of lions, natural selection and genes, and the impact of man on lions in wildlife parks. Apart from a few solecisms, Dr Bertram writes well.

In *Tiger! The Story of the Indian Tiger* (Collins: London, £7.95), Mr Kailash Sankhala deals similarly with the tiger, but also includes much personal material—as he says, the book might be called the autobiography of a tiger addict. His formal training was not in zoology but forestry; after postings to various Forest Divisions of India, and managing the wildlife sanctuaries in some of them, he took charge of Delhi Zoo in 1965 where he remained for five years. Since then he has made a census of the wild tigers of the subcontinent, which resulted in the setting up of over a dozen tiger reserves to conserve the dwindling numbers of tigers and other animals. The first part of the book describing the natural history of the tiger, and the author's adventures in studying it, is the story of an enthusiastic naturalist enjoying the outstanding richness of India's wildlife. The author discusses, from his extensive experience, the tiger's solitary daily life, its environment, prey, co-predators and camp followers, and the other wildlife, and makes a very good job of it.

In the second part of the book Mr Sankhala considers matters such as hunting, circuses and zoos, the near extermination of the tiger and the efforts made to preserve the species. He also makes a lengthy apology for

man-eaters and cattle-lifters, and adds some snooty remarks about the exploits of Jim Corbett in killing them. Nevertheless, tigers do sometimes kill and eat people. He becomes really controversial, however, when he talks about zoos and circuses, both of which he condemns as outmoded and no longer justified. When he went to Delhi Zoo he soon saw that its main role was not education but entertainment, with "riding elephants, begging bears, performing otters, smoking chimps and talking parrots . . . and the Children's Zoo a mere toy house". He did away with all this in spite of the unpopularity it brought upon him, and developed the zoo as "a wilderness with minimum signboards and direction indicators". Yet even though rare animals bred so



Sick baby orang-utan rescued from an animal dealer

that he was proud of his success and convinced temporarily of "the importance of zoos in conservation", at other times he felt he had more blood on his hands than the most hardened poacher. He points out that zoos can never play much part in conserving rare species, and the craze for safari parks and zoos is not the answer to saving them. "Now that ecology, environment and the biosphere are all the rage they are suddenly changing their signboards to become 'Ecological Centres' or 'Biological Parks'. The new zoos are designed on an environmental concept", but even with the best designing they come nowhere near this ideal—even the best zoos are travesties of nature that mock their inmates. "The whole idea of bringing a free-living animal into captivity is revolting, and simply because they eat well and reproduce in captivity does not mean that a zoo is a proper environment for them".

As someone has said, a zoo is a lot of wild animals kidnapped from their native freedom and locked up for un-

comprehending yahoos to gape at in a sordid slum where they pitifully try to fulfill their innate behaviour patterns. The author points out that wildlife films seen by millions on television are far more educational than animals in zoos, and asks "if it is not necessary to bring the Grand Canyon or the Taj Mahal . . . into your city why should there be any justification for netting and snaring animals for educational purposes at a zoo? . . . Except for a few zoos which do a real job of contributing to knowledge their objectives are pure hypocrisy". Anyone who knows anything about the way zoos are run will, if he be honest, heartily agree. The proper place for wild animals is in the wild.

Mr Sankhala, like Dr Bertram, thinks that nature reserves as alternatives to zoos should be run with the minimum of "scientific" management. Mr Guy Mountfort, in *Back from the Brink* (Hutchinson: London, £5.50) tells of the making of some reserves, and of the ways by which several 'endangered' species have been saved from apparently imminent extermination. He is a dedicated supporter of the 'conservation movement' who draws a picture of himself as the super dogooder, trotting about the globe addressing meetings, button-holing VIPs—he seems to like dropping names—raising money, and intriguing to get official approval for reserves and animal parks. His material is interesting, but his style of writing is that of the establishment and the old boy network—the man in Whitehall knows best.

Mr Christian Zuber, on the other hand, is a cameraman who specialises in wildlife films that are mainly shown by French television, on which he is a public figure. He, too, is an ardent conservationist but no zoologist; he consequently makes some surprising blunders in his statements about animals in *Noah's Ark: Animals in Danger* (Cassell: London, £8.95), but perhaps some of them are due to his translator. His pictures, however, are excellent, informative and interesting, though some of them cover the same subjects as those in *Back from the Brink*. It is strange that conservationists should write books with such good illustrations but such boring texts.

All these authors are much troubled that the wildlife of the world is rapidly decreasing under human depredation. The causes of world decline are many, some are unavoidable but others unnecessary and disgraceful. Foremost is the expanding human population, steadily encroaching on the wilderness to satisfy the hunger for land and what it may produce. The mad rush by Indians to cut down the forests and shoot all the animals when law and

Taken from *Back from the Brink*

order broke down at independence was typical. A second cause is the commercial value of wildlife products that leads to ruthless exploitation; apart from whales, there is the trade in turtles and their eggs, the gathering of colonial-nesting sea birds' eggs on a vast scale, the poaching of ivory and rhinoceros horn secretly condoned and participated in by corrupt administrations in Africa, the killing of crocodiles and lizards for the luxury leather trade, the hunting to supply the demands of the fur trade with pelts of all kinds, from vicuña to tiger, leopard and other large cats. But international agreements to protect some species and control the trade in their products are now beginning to have some effect.

The worst threat to wildlife, however, is the development of remote places for the tourist trade. Remote and beautiful places are disfigured with airports and huge hotels to which hordes of tourists are brought on package holidays. The once placid countryside is invaded by speed boats and water skiing, spear fishing, discos, noise, disturbance and destruction of habitat. The traders in eagerly bought souvenirs are ransacking the remotest shores of the tropics for rare sea-shells, despoiling island faunas to provide stuffed baby crocodiles and iguanas, and encouraging the poachers to supply animal skins and carved ivory. Wildlife parks where animals are preserved provide no safeguards—tourists may be valuable money spinners, but they have to be housed, air-strips and roads must be made to get them there, electricity, water and sewage works supplied, catering organised, the animals 'man-

aged' by wardens, and so on until the whole thing is as artificial as a 'safari park' in a developed country; the tourist industry is a vast humbug. Guides take the tourists to photograph the animals—Dr Bertram in his land-rover once found he was followed closely by eight minibuses and cars full of tourists, and he gives a photograph of a tourist minibus, with camera snappers, harassing a lioness at close quarters. Mr Sankhala also remarks that the snap-shooting tourist is so preoccupied with shutter speeds, lens apertures and focussing that he fails to see anything around him, and adds that a visitor to a reserve should bring nothing with him but a receptive mind and take nothing away but what it receives.

It is inevitable that, in spite of the craze for wildlife, within a hundred years the wilderness will be tamed, the larger wild animals that have not become extinct will be contained within fences, and development will have destroyed the spirit of freedom. The only way to preserve samples of the fauna is to isolate reserves in which no human disturbance is allowed, such as the area between Seronera and Lake Victoria that formerly was a closed reserve free from human footprints or car tracks. But will the developers agree to large parts of their countries being sterilised against their enterprise? The call of money is louder than the voice of the wilderness.

L. Harrison Matthews

L. Harrison-Matthews was Scientific Director of the Zoological Society of London from 1951-66.

Protean nature of conservation

THREE of the four books reviewed here (that of Maurice Burton is the exception) could come under the heading of conservation but their diversity emphasises the protean nature of the term. Definitions are many and various (if ventured upon) but I know none so comprehensive and thoughtful as that of Charles Elton in his *Ecology of Invasions*, viz. "some wise principle of coexistence between man and nature, even if it has to be a modified kind of man and a modified kind of nature".

These three recently published books, among many others, suggest that, at the moment, the lion's share of this modification has fallen to nature while man is panting behind. Richard Williamson's *The Great Yew Forest: The Natural History of Kingley Vale* (Macmillan: London, £5.95) is a fascinating and first-hand account of his struggles, as warden of Kingley Vale National Nature Reserve, to understand and maintain the unique

yew forest with its interspersed chalk grasslands; it shows how hard it is to stem the influence of man even in a nature reserve. It is a first-rate, thoughtful naturalist's book and will be enjoyed not only for its vivid observation but for its searching out of natural processes.

In contrast, Richard Perry's *Wildlife in Britain and Ireland* (Croom Helm: London, £7.25), published in association with the World Wildlife Fund, is avowedly a compilation about the history of the bird and mammal fauna of the British Isles since the last Ice Age. It gives special attention to the social pressures that have, for example, eliminated wolves and bears and subsequently borne hard on predators generally and, by contrast, allowed the penetration by exotic species such as the muntjac and sika deer from patrician zoological collections or nutria and American mink from commercial drive. There is some evidence of the beginning of a modified kind of man in his attitude to nature in that animals are less regarded as simply subserving his needs or pastimes. Mr Perry's collation of the prehistory of

An Introduction to Animal Behaviour

Third Edition

Aubrey Manning

In this new edition, the main aim has been to keep a broad biological approach to the subject. There has been a considerable amount of rearrangement of the material to take account of new attitudes to behavioural problems. Thus, for example, imprinting is now considered under the discussion of development, and the contents of the hitherto separate chapter on hormones and behaviour, are now considered with those on development and motivation where they are most relevant. Sections on communication and on sociobiology are the most important new additions and serve to introduce students to some of the results and concepts in these active research areas.

Cloth £12 Paper £5.95

Organization in Plants

Third Edition

W. M. M. Baron

This is the third edition of a very successful text on plant physiology for both introductory biology and botany courses at universities and in sixth forms. The book has been extensively rewritten to keep abreast of recent discoveries. Much more information on the growth and development of plants is included to stress that the survival of the plant as a species may depend on all stages of its life cycle.

*With illustrations throughout
Paper £6.75*

Micro-organisms

Function, Form and Environment

Second Edition

**Edited by Lilian E. Hawker
and Alan H. Linton**

This second edition of a highly acclaimed text has been largely rewritten and re-structured to take account of recent developments in the field. The editors have organized the material around the three main themes of physiology and biochemistry, natural history and classification, and interaction with the environment, to produce a logical and readable text covering the whole field of microbiology.

*With illustrations throughout
Paper £8.95*



Edward Arnold

41 Bedford Square,
London WC1B 3DQ

our fauna is valuable, even if the details are not minutely documented. I could detect no serious errors, though it was startling to find (p63) the strawberry tree flourishing at 10,000 feet in the Alps. This, presumably, refers to the alpine bearberry (*Arctostaphylos alpinus*), which was previously put in the genus *Arbutus*. The great value of the book is to give us some measure of the mutability of the things we try to conserve quite apart from the ever-increasing pressure exerted by the numbers and activities of man.

A quite different aspect of conservation is treated by Lawrence Alderson in his book *The Chance to Survive: Rare Breeds in a Changing World* (Cameron and Tayleur/David and Charles: Newton Abbot and London, £7.50)—namely, the urgency of preserving the breeds of horses, cattle and pigs which, having gone out of fashion, are in danger of being permanently lost. I would not have thought it possible that, being a straight zoologist, my interest and enthusiasm could have been aroused by a theme so much on the fringe of my usual thinking. But Mr Alderson writes so well and with such conviction that I found myself carried along and taking in my stride the welter of British Whites, North Ronaldsays and Gloucester Old Spots (shades of Michael Innes!). His main contention is that, nowadays, breeders of livestock are too concerned to concentrate on specialised characteristics which suit the immediate market, especially in the conditions of indoor cossetting. So breeds that are thrifty and can live on marginal land tend to die out. Yet these may be just the ones that will be needed when environmental and economic conditions change. The book is a plea that such breeds should be regarded as valuable gene banks and it points to how much has already been achieved by the enthusiasm of recently formed breed societies and by the formation of the Rare Breeds Survival Trust. Darwin would have found this book a worthy extension of his *Animals and Plants under Domestication*, especially as so many of the principles of genetics, which were a closed book to him, have now been unravelled. Nevertheless, the impression conveyed by this book is that there is still a long road to travel before the complexities of inheritance in our farm animals are fully elucidated.

These three books, therefore, illustrate forcibly what I have referred to above as the protean nature of the concept of conservation: yet all have some common aim—that of preserving faunal diversity. Kingley Vale is a unique ecosystem and it is good to know that its ancient yew forest is safe from despoliation and cared for by a warden who wants to know “what makes it tick”. The problems of management of the British vertebrate fauna, involving so many divergent interests from those who practise agricultural monocultures to those who cannot bear to see blood

spilled cannot even be approached without an evaluation of its present status and past history, such as Richard Perry has tried to provide in his book. Finally, the stock-taking of farm animal breeds and the plans that are being put into action to deep-freeze semen and ova of vanishing strains, whose qualities may be urgently needed in a cloudy future, must counteract the gene pools that, once vanished, are gone for ever. Thus, we hark back to “a modified kind of man and a modified kind of nature”. The main kind of modification of man so far exhibited is a deeper appreciation of and concern for the preservation of diversity and richness of ecosystems, habitats, flora and fauna. This is an aim on which, in this country, the Nature Conservancy Council and its allied research branch, the Institute of Terrestrial Ecology, have always concentrated their energies. It is, and will be, an uphill fight until the pressure of man

and his activities is lightened—one hopes by planning in the best sense and not by catastrophe.

The last book of the quartet, *Just like an Animal* (Dent: London, £5.50) by Maurice Burton, is just like Maurice Burton—a concatenation of anecdotes about animal behaviour ranging from that of ants to that of his own, and other people's, pets. The theme is altruistic behaviour in animals and many of the episodes quoted are, as Darwin said about the natural history of the Galapagos Islands, “eminently curious”, though they do not add up to such far-reaching conclusions.

H. N. Southern

H. N. Southern recently retired as Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford, UK.

For herpetological novices

THE latest of the Collins' *Field Guides*—*A Field Guide to the Reptiles and Amphibians of Britain and Europe* (Collins: London, £4.95) by E. N. Arnold and J. A. Burton—is a perfect gem, a delight to the eye and a pleasure to handle—a fine, solid, book with a well designed and illustrated, waterproof cover. In Europe, west of 36E, there are about 45 species of amphibians and 90 species of reptiles, including five sea turtles, all of which are described and illustrated with the aid of numerous diagrams, 126 distribution maps, and 40 superb coloured illustrations from drawings by D. W. Ovenden. It really does show how dreadfully the British fauna must have suffered from extinctions during the Ice Age—I can hardly wait for our next holiday in Europe.

The book contains some delightful touches of realism which must surely appeal to professional zoologists as much as to absolute novices. For instance, the following approach to identification is recommended—first, “try to match your animal's appearance as closely as possible” to one of the species shown in the plates. Then “check with the text and maps that you are within the range of the species concerned”. “If the range fits, consult with the description in the text and, lastly, if all else fails, try using the keys”. No hypocrisy here about using keys first, and then checking the descriptions and illustrations.

It is suggested that a photographic record be made of reptiles and amphibians seen, but the keeping of pets is discouraged. “The average semi-moribund captive tortoise bears no comparison with the alert, vigorous, herb-scented

beasts that plough through the undergrowth of southern Europe. Observing wild animals is far more entertaining, and less demanding, than the drudgery of keeping them”. All interesting animals found dead can, however, be preserved, and the reader is informed that, in emergencies, quite good results may be obtained with “the stronger grades of brandy, gin, anis, slivovica, tuica, rakija, schnapps, or ouzo”. Yet another advertisement for a continental holiday.

The World of Amphibians and Reptiles (Sampson Low: Maidenhead, UK, £3.95) is a second attractive volume for people with herpetological interests. Although translated from an Italian original it is quite well written. Indeed, it seems both surprising and unfortunate that the author's name should have been withheld. When reading a book, I prefer to know whose ideas I am absorbing. And although, presumably, written mainly for students and amateurs, the book under review does propound some genuinely interesting ideas as well as others that seem rather odd. For instance, amphibians can tolerate cold better than reptiles, especially in regions where the climate is dry: many Hynobiidae are to be found in the cold, but muddy, tundra regions on the Siberian border. The Plethodontidae “have a fixed mandible and in order to chew or swallow they have to move their jaws, and with them the entire cranium”(?). Nocturnal Bufonidae move with small, clumsy hops, as their hind legs are very short—the causal relationship, if any, is not, however, elaborated.

Interesting comments and speculations are made as to the functions of cloacal and muchal-dorsal glands in snakes, predation by land tortoises, the pineal eye of *Sphenodon* and lizards, egg-teeth and caruncles, the sticky membrane of some chameleon eggs which gives them a hold on a branch, and stomach stones

of crocodilians. We are also told that snakes "spend most of their time half-asleep, and the only stimuli that can rouse them from their state of torpor are hunger-pangs (due to a previous period of fasting, due in turn to laziness) and sex".

On the other hand, there is no serious discussion of the physiological significance of cutaneous respiration in Amphibia; the origin of the cleidoic egg is barely mentioned; adhesion of the gecko's foot to smooth surfaces is explained without reference to friction; the ability of *Chrysopelea* to glide is stated without comment; and physiological thermoregulation in reptiles barely mentioned. The problem of the coloration of coral snakes is not analysed and no reference is made to Mertensian mimicry in this context. Nor can I accept the argument that, as the colours of chameleons are most cryptic when the animals are relaxed, their function is not so much for defensive reasons, but rather to give them a chance to attack a prey unseen.

Portraits of the Lepidoptera

"KILL not the moth nor butterfly" wrote Blake, and three recently published books reviewed here echo his sentiments, though they are in defence of conservation (or just sheer artistry) rather than because "the Last Judgment draweth nigh". All three have superb illustrations and none refers to the killing bottle or setting board.

Robert Goodden's *British Butterflies: A Field Guide* (David and Charles: Newton Abbot and London, £4.50) is perhaps the best organised. It really is a field guide, and starts with a section on the life-cycle, breeding and conservation of British butterflies, and one can see from it how much there is still to be learnt about their habitats. Every species is photographed alive and with each there is a rough distribution map. For the beginner difficulties of size are overcome by the charts at the front and back of the book, which show the insects (the common ones at the front and the rarer ones at the back) at approximately three-quarters their size. There is advice on how to encourage butterflies to visit gardens and an exhortation for the enthusiast to join the British Butterfly Conservation Society in Leicestershire. The book is for the uninitiated of all ages, and for the old hand is a "must" simply for the breathtaking photographs.

Umberto Parenti, in *The World of Butterflies and Moths: Their Life-Cycle, Habits and Ecology* (Orbis: London, £5.95) looks at some of the many facets of the world of butterflies and moths. The photographs are of unbelievable beauty, and it seems churlish to have to criticise them by saying that they are arranged

The two sections into which the book is divided are provided with introductions which explain clearly how each class has evolved to its present status. These sections are followed by accounts of structure, physiology, reproduction, behaviour, ecology, and classification. A detailed bibliography of relevant books is included. The text of some 60,000 words is illustrated by over 200 colour photographs and drawings, but the latter are not always related to the text and are of inferior quality to those of the Collins' *Field Guide*: some are distinctly crude and inadequately labelled. The index too is incomplete. Despite such comparatively minor inadequacies, the book provides an excellent introduction to amphibians and reptiles and can be confidently recommended as a Christmas present that will give interest and pleasure.

J. L. Cloudsley-Thompson

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often randomly, both geographically and for the text, and that there is no indication of the actual size of the insects. The book is at its best in the information which it gives about the evolution of the Order, its anatomy and physiology, the methods of reproduction and the way the insects adapt to their environments, with sections on mimicry, camouflage and pheromones. The least satisfactory section is the classification, which is difficult to take in, and being necessarily short is over-compressed for such a vast subject. In spite of some unevenness the book is strongly recommended, and would make a splendid Christmas present.

Stuart McNeill's examples of British butterflies and moths, in *Butterflies and Moths* (Michael Joseph: London, £11.95) have been selected from the famous series of Christian Sepp and his son, the engravings first being published in Holland in 1762. The introduction explains the technique of copper engraving and gives a history of the Sepp family. The text relating to the insects is up to date, and one notes that the Peppered Moth was true to its name in the Sepps' day—the "dark, Satanic mills" which were to bring industrial melanism being still in the future. The book is recommended more for bibliophiles and nature lovers than for either beginners or specialists in the Lepidoptera. The restrained beauty of the plates makes the exuberance of the colour photographs in the other books seem vulgar, and yet it is interesting how accurately both can portray the insects.

Cyril A. Clarke

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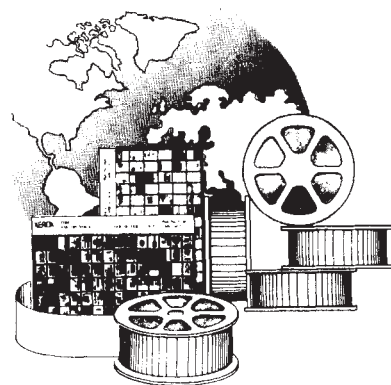
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Natural selection in wildflower books

WILDFLOWER BOOKS in Britain are now proliferating at a rate which almost matches that of bird books. This is indicative of a large and growing market among adult amateur naturalists as well as among the more enthusiastic element within school and university classes. It is not easy to classify flower books, but one might begin by dividing them into field guides, the chief intention of which is to aid the process of identification, and coffee-table books, which rely on the aesthetic appeal of elaborate photography or artwork to attract a market. On thumbing through a selection of recently published flower books one soon realises that this dichotomy is unreal and that most books actually lie along a continuum of variation between these two extremes.

In Britain we have been well supplied with illustrated field guides, in particular D. McClintock and R. S. R. Fitter's *Pocket Guide to Wild Flowers* (Collins: London, 1956) and W. Keble Martin's *Concise British Flora in Colour* (Ebury: London, 1965). Both of these sought to provide comprehensive coverage of the British flora in the form of illustrations and notes on identification and distribution. Many taxonomic botanists have been unhappy with the development of such books, for students are apt to use them uncritically when identifying plants and prefer to look through pictures rather than work through keys. Undoubtedly such misuse of these books does occur, but on the other hand illustrations can be most valuable in confirming identifications and in exciting the interest of incipient naturalists. On this ground alone, in my opinion, they justify their existence.

In a similar vein to these earlier works is *Wild Flowers: The Wild Flowers of Britain and Northern Europe* (Collins: London, £5.95) by M. Blamey, R. Fitter and A. Fitter, which is now available in a large format, hardback edition having previously been published as a paperback pocket-sized book. Illustrations are now, unfortunately, separated from the text and although a taxonomic order is adopted, only English names appear on the plates. This is a great disadvantage over the Keble Martin text, especially where students are concerned. Some of the English names used are not entirely satisfactory, such as 'dwarf thistle' for *Cirsium acaule*, instead of the usual 'stemless thistle'. Some habitat details are inaccurate: *Dianthus superbus* is said to be found in dry places; in Fennoscandia it is found largely on riversides. Problems have arisen as a result of the separation of illustrations from the text (they were interleaved in the paperback edition); there are errors in cross-referencing and those species which were mentioned but not illus-

trated in the paperback are now omitted entirely. This means that the book is far less comprehensive (such a common species as *Cirsium palustre* is lacking) in its coverage.

The justification for this new format edition with reduced text is the desire to display Marjorie Blamey's illustrations to better effect. This is certainly achieved and the plates are of a high quality. One must, however, compare them with those of Keble Martin and they fail to measure up to the earlier paintings on either aesthetic or technical grounds. Take, for example, the pictures of *Eriocaulon sept-angulare*; in Keble Martin's illustration the flower structure is clearer, the grooved, spiral stem is visible and the cell structure of the leaves is evident. All of these are useful criteria for identification and are lacking in Marjorie Blamey's work. It is only fair to add, however, that recent editions of Keble Martin's drawings have deteriorated in their quality of reproduction.

Roger Phillips' *Wild Flowers of Britain* (Pan: London, paperback £4.50) approaches the illustration problem by means of a series of colour photographs, yet it remains essentially at the 'field guide' end of the spectrum. The approach has been used quite successfully in the past by Oleg Polunin in such books as his *Flowers of Europe* (Oxford University Press: Oxford, 1969). There are two novel features in Phillips' book: the plant material in the photographs is picked and arranged in a studio setting. The photographs are most effective and the freshness of the specimens is extraordinary. One could object to the technique on conservation grounds but rarer species such as *Gentiana verna* and *Dianthus gratianopolitanus*, are either represented by garden specimens, or habitat shots are used which do not necessitate the destruction of the plant. The other novel feature of the book is the arrangement, which is based upon flowering time, and where appropriate it includes fruit in due season. The coverage is quite full, including some non-native species such as *Cornus mas*, and the mixing of illustrations with text makes it easy to use. This is a valuable contribution to the popular botanical literature, though I still prefer the drawings of Keble Martin.

The Hamlyn attempt to invade the field guide market with Helen Pursey's *Wild Flowers* (Feltham, UK, £1.50) falls short in every respect except its low price. Only 220 species are dealt with and yet the geographical coverage includes northern Europe. The selection of plants included seems to be random; for example, the only species of *Melampyrum* described is *M. cristatum*, which is probably the least likely species of the genus to be encountered in northern Europe. On these grounds alone it is more likely to frustrate than to satisfy or inform the beginner.

Dent

Collins (London, £5.95) has recently taken the daring step of issuing *An Atlas of Wildflowers of Great Britain and Northern Europe* compiled by Alastair Fitter, consisting of almost 2,000 maps of species distribution in northwest Europe. It is a daring step in that such a book cannot hope to have the immediate appeal of the colour picture book, yet it must be regarded as one of the most valuable contributions to popular botany of the decade. Apart from the obvious use of such maps in plant hunting, the enthusiast who has learned basic plant identification from the field guides will now be able to consider what environmental factors, such as climate and human modification of habitats, are instrumental in the production of the distributions described here. It should also provide a more balanced attitude to conservation of the British flora, for, although such glamorous species as *Cypripedium calceolus* are bound to attract much effort on the part of preservationists, some of the most distinctively 'British' plants are the oceanic heathland types such as *Ulex gallii*. This realisation may increase public awareness of the need for the conservation of these internationally scarce habitats which we rather take for granted.

Lys de Bray's *The Wild Garden: An Illustrated Guide to Weeds* (Weidenfeld and Nicolson: London, £6.95) concentrates on weed species and falls closer to the coffee-table end of the spectrum than the field guide end. It is very much a folk lore type of book with an abundance of legendary material and herbal recipes. John and Susan Proctor's *Nature's Use of Colour in Plants and Their Flowers* (Peter Lowe/Eurobook: London, £3.95) also gives an initial coffee-table impression, especially in its lavish full-page colour illustrations of flowers like *Strelitzia* and of fruits like the apple. It does, however, go far beyond this level in discussing the nature and function of plant pigments, and of pollination mechanisms. A chapter on plant mimicry is particularly valuable, as this is a subject rarely dealt with in popular texts. Both Batesian and Mullerian mimicry are explained and illustrated from the plant kingdom. Parts of this book could be read by sixth-form (advanced school) students and first-year undergraduates with great benefit.

North American plant books are represented in this list by a field guide, *Wildflowers and Weeds*, by B. Courtney and J. Zimmerman (Van Nostrand Reinhold: Wokingham, UK, paperback £5.30) and by a coffee-table book, *Wild Flowers of Canada*, by M. Ferguson and R. Saunders (Van Nostrand Reinhold, £15.15). The latter contains some superb colour photographs of plants arranged in habitats and links these to brief, if prosaic, text descriptions. With only 150 species described it does not provide adequate information for identification. *Wildflowers and Weeds* consists of a series of small

colour photographs covering 650 species from the Great Lakes area. Arrangement is taxonomic and text notes are reduced to a bare minimum. The small size of the photographs and their poor quality of reproduction limit the value of this book, which otherwise emulates the approach of Polunin in Europe. It presents no challenge to the Tory Peterson guides in North America.

Finally, there is a coffee-table book by W. P. A. Jackson, *Wild Flowers of Table Mountain* (Howard Timmins: Cape Town, £12.95), which has some excellent photographs of flowers growing in their natural setting on Table Mountain, South Africa. The text is informative and interesting and should appeal to horticulturalists with a penchant for the Cape flora.

Orchid guide

A Field Guide to the Orchids of Britain and Europe with North Africa and the Middle East. By J. G. Williams, A. E. Williams and N. Arlott. Pp.176. (Collins: London, 1978.) £4.95

SOONER or later, most amateur botanists reach a point the popular, general floras seem not to describe their latest discovery, while the specialised works are too bulky, too costly, or only available in a library. Those whose problem lies with identifying wild orchids, not only in Britain, but virtually anywhere within reach of the home-based holiday traveller, should be frustrated no longer, for this new field guide ranges geographically from the Azores to the Urals, by way of Iceland and the countries of the Maghreb. It describes and illustrates 138 species, as well as a large number of sub-species and varieties, is small enough to go in the pocket, and is very reasonably priced.

The lay-out is convenient, each description being faced by the relevant illustration, although the very small type may prove a problem for those whose sight is not what it was. A few introductory paragraphs are followed by a key to the genera—which seems a trifle unnecessary—and more detailed keys to *Ophrys* and *Orchis*; the authors have wisely not attempted a key to the complex genus *Dactylorhiza*. In general, the *Flora Europea* classification has been followed, but it is unfortunate that means of correlating this with the nomenclature adopted in older floras are not provided. Descriptions of the individual species are in general sufficient and accurate, although there may still be some frustration over sorting out the scarcer Mediterranean species of *Ophrys*, and, inevitably, the *Dactylorhizas*. Some mention might have been made, too, of the many interesting, puzzling and not uncommon inter-generic hybrids which are features of the family as a whole. One might also query some of the authors' assessments

The radiative evolution of flower books seem to be operating on a number of major lines. Photographs are taking over from detailed and accurate artwork, which is unfortunate. Some attempt is being made to provide information over and above that which is relevant to identification; this is to be welcomed. What needs to be developed is the use of simple keys to add precision to the identification processes (for example, with the yellow Composites) and the placing of plant species into habitat contexts so that their interactions can be better appreciated.

Peter D. Moore

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Oncidium lanceanum (Orchidaceae)

Mary Evans Picture Library

of the rarity of certain species, and their data on distribution are not always as complete or up-to-date as they might be.

The illustrations are perhaps somewhat less satisfactory. At least as here reproduced, they do not reach the high standard attained in other books in this series, and it is at times evident that, as is made clear in the Introduction, the artist has worked from the authors' sketches, and has never seen the living plant. Considerable attention is given, too, to enlargements of parts of no particular value as aids to identification by the ordinary amateur. Two excellent features that follow the main text are an unusually full glossary, and a check list wherein the reader can record his or her own personal finds. In general, these are minor criticisms of points easily rectifiable in a later edition; they certainly do not detract from the general usefulness of a book, which fills a notable gap in the amateur botanist's library. **Peter Collins**

Peter Collins is a freelance science writer who has been studying the orchids of Europe for more than 25 years.

Armchair geology

THE mere statistics of wilderness areas are impressive enough. The Earth's surface contains something like 18×10^6 km² of desert, about half of which is accounted for by Australia and the Sahara. And to this 12% of the total land surface must be added 11% under permanent ice cover, 9% covered with mountain, a further 9% of cold and waterlogged tundra, and 29% of otherwise uncultivable continent comprising semi-arid regions, bare rock, frost-prone areas, and so on. Taking the oceans into account now, only 9% of the Earth's surface can be cultivated and only about a third of that is actually tilled.

To be able (by courtesy of the BBC) to escape from man's narrow well-beaten tracks from time to time to make a series of television programmes about the world's varied wildernesses, Anthony Smith, the naturalist-broadcaster (as opposed to the political scientist-broadcaster and the medical journalist of the same name), was indeed privileged. His book, *Wilderness* (Allen and Unwin: Hemel Hempstead, UK, £7.50), though obviously a spin-off from the series, is not the "book of the film" in the usual sense but, rather, an independent personal account involving a mixture of description, observation, reaction, interpretation, background and anecdote relating to his visits to the world's largest ice-sheet (Antarctica), the biggest tropical forest (Brazil), a large desert (central Australia), the highest mountain range (Himalayas), the tundra (northern Canada) and the most extensive swamp (Sudd).

"Discovering a nest", says Smith at one stage with unconscious irony, "is more rewarding than being shown ten of them." Precisely—which is why all travel books are inherently unsatisfactory. Whether you like or dislike this one will depend on whether or not you can bear to visit places through another person's words. Generally I cannot; but because of the unusual nature of the places, I managed to overcome the resistance in this case and was rewarded with a pleasant afternoon, not least because of the superb colour plates.

But if Smith's book is the closest most of us are likely to get to the true wilderness, the phenomena described by P. J. Banyard in his *Natural Wonders of the World* (Orbis: London, £5.95) are more accessible, as they lie mainly within man's proportionally small terrestrial domain. Landforms, ice patterns, water displays, atmospheric phenomena, gurglings from the Earth's interior, exotic flower arrangements—they are all here in a combination of pleasing pictures and straightforwardly factual text. It makes for a nice browse; but there is a sense of *deja vu* about it all, for what we are looking at is just the

latest version of the world picture book that grandma bought us for our eleventh birthdays. There is little one can fault Banyard on, lest it be lack of inspiration; but it is at times like this that one comes to appreciate Anthony Smith's sense of personal quest and involvement.

Involvement of another kind is inherent in Alan Woolley's *Illustrated Encyclopaedia of the Mineral Kingdom*, (Hamlyn: Feltham, UK, £5.95) for mineralogy in its simplest form, the collection of minerals, is the most popular of geology's branches, at least as far as the amateur is concerned. I presume that this new Hamlyn encyclopaedia has been produced largely with the non-geologist in mind, but non-mineralogical Earth scientists will also find it useful as far as it goes. Its main defect as far as working geologists are concerned is that it only goes as far as about 300 of the world's 2500 or so minerals (generally the most common and widely distributed but including a few rarer ones of greater than usual interest to mineral collectors).

For the chosen 300, however, all the essential information is given (composition, hardness, lustre, occurrence, and so on) and the colour photographs are good. On the other hand, the book is far from being a mere list, for there are summary chapters on the Earth's structure and history, crustal composition, minerals and rocks in their geological environ-

ments, crystals, gemstones, economic minerals and the building of a mineral collection. This book too is but a variant of its numerous predecessors over the years; but within its stated limitations it is far more authoritative and better produced than most.

Aggregates of minerals (that is, rocks) are generally less colourful than some of the pure minerals and thus less popular as collectors' items. The most colourful specimens in *Rocks of the World*, (Pergamon: Oxford, £15), which is not a book but a neatly-mounted set of 36 of the world's most basic rocks, are the laterite, the serpentinite and the ignimbrite; but despite the preponderance of greys the collection is a fascinating one, not least by the way it suddenly appears in one's hand without all the effort of collecting individual samples. Armchair geologists of the world will rejoice; but schoolteachers will not be displeased either, for a good collection of basic rocks is, of course, essential for beginning geology classes. The samples are rather small (up to about 4 cm across) and, being mounted, cannot easily be handled by a group of students. As a purely visual display, however, the set can hardly be faulted.

Peter J. Smith

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For budding holographers

Understanding Holography. By Michael Wenyon. Pp. 176. (David and Charles: Newton Abbot and London, 1978.) £5.50.

THIS book is intended for the general reader and starts with two chapters about light and the laser in which various ideas relating to waves, coherence, interference, stereoscopic images, stimulated and spontaneous emission, and so on, are introduced in a non-mathematical manner. There follows a short digression on the applications of lasers in such fields as medicine and laser light shows before a discussion of the principles of holography. The basic ideas of holography are considered from the diffraction grating approach, and a well informed reader may have thought that more insight into the process would have been obtained, had the idea of the grating been extended to include the Fresnel zone plate. The effects of the size and spectral content of the light source used in the reconstruction process are, surprisingly, not considered in detail.

The chapter on display holography makes entertaining reading and there are many illustrations (necessarily two dimen-

sional) of what has been achieved in this field. It is possible to make holographic pendants, three dimensional abstract light patterns and rainbow cylinder holograms; and even Salvador Dali has shown an interest in the technique. As for the future of holography, the author tells us that holographic movies already exist and could well become a viable proposition on a large scale. There is, however, a sinister comment that "life-like 3D movies could also be used in the future for psychological conditioning or brainwashing". Holographic television, it seems, is unlikely to be realised in the near future. Apart from its obvious entertainment value, holography can be used in a more serious vein, as illustrated by the accounts of applications in the study of the vibration modes of musical instruments and the testing of car tyres.

The final chapter is an account of how to make your own holograms with simple equipment. Perhaps the easiest way of making a simple hologram is to use conventional fine grain film and to ensure there is a small angle between the object and the reference beam. It is a pity there is no mention of this experiment, if for no other reason than the relative cheapness of the photographic material. Also the double exposure scatter plate experiments could have been described as they are easy

to do with conventional photographic film. The final sentence is a warning never to look directly into a laser beam. Perhaps there should also have been a suggestion that the budding holographer ought to consult an expert before he purchases any

equipment. It is so easy to labour in vain.

A. Thetford

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Astronomy for the amateur

ASTRONOMY is a subject which lends itself to the lavish illustrator. On the one hand you have the photographs, a plethora of nebulae, galaxies, star fields, planetary surfaces and lunar landscapes; on the other, you have the imaginative artist only too keen to paint what the eye cannot see (or being ever the optimist I should say "hasn't yet seen"). And with figures, scale diagrams of satellite and planetary systems, galactic clusters, binaries, stellar cross-sections, geological and planetological features, the list could be enormous. I'm a great believer in the adage that a good picture is worth a thousand words and it is obvious that many authors of astronomy texts are too. Looking through some old and some not so old books for the astronomically minded layman, one obvious change with time is the ratio between diagrams and text. In the three astronomical 'glossies' I have before me now this ratio seems to be way above 70%. And why not? What an enchanting feast for the eye they are. The best is by Peter Ryan and Ludek Pesek (*Solar System*, Allen Lane: London, £9.95). Ryan's text gives an easily understood accurate and up-to-date account of our knowledge of the Sun, the planets and their satellites. Pesek's paintings are absolutely fascinating, magical insights into Venusian clouds, Saturnian rings, Jovian electrical storms and a host of planetary and satellite landscapes, all with a starkness that is breathtaking. The photographs are well chosen and beautifully reproduced. But special praise must go to the studio (QED) who produced the diagrams; these are superb. This book is an enchanting guide to the interplanetary age.

Dropping the price by a factor of three we have Ian Ridpath's *Stars and Planets* (Hamlyn: Feltham, UK, £2.95), a lavishly illustrated, rather thin, romp through the history of astronomy, the Solar System and the Universe. On a price per page basis, it is comparable to Ryan and Pesek, but I like Ridpath's style. It bubbles along infectiously and makes this book an ideal introduction to the wonders, beauties and new things in astronomy.

My final big book is a revised edition of the *Mitchell Beazley-Patrick Moore Atlas of the Universe* published in 1970 (*The Mitchell Beazley New Concise Atlas of the Universe*; Mitchell Beazley: London,

£12.95). The blurb stresses the fact that it is "new" and "completely revised", but on reading the book one gets an impression rather like opening a packet of "new" "improved" soap powder. The contents differ little from the previous. Not of course that this is altogether a bad thing. The 1970 edition was very good, and these good features remain in this new edition: the star maps, the moon maps and the potted descriptions of a host of astronomical objects. The planetary sections have obviously changed the most and they contain results from all the recent space probes. The book is an excellent aid to learning, ideal for the new astronomer and the schoolroom. If you haven't got the first edition then I recommend this book heartily. If you have the first edition, stick to it and save your money.

Staying with the younger reader for a while we have *Astronomy: An Introduction* by Jacqueline Mitton (Faber: London, hardback £6.25; paperback £2.75). This small format book has about 120 pages of text and its audience seems to be scholars taking the Ordinary Level (middle school) Astronomy examination. Its main usefulness seems to be in what it leaves out. Here is what you would need to know to pass the exam—no more, no less—so it is ideal for a busy scholar but is rather skeletal for others.

The plaintive knockings of Walter de la Mare's horseman are echoed in my next two books. As the Earth-bound traveller, or should I say a few of our travellers, call out to the Universe, "Is there anybody there?", Iain Nicolson (*The Road to the Stars*, David and Charles: Newton Abbot, UK, £5.95) encourages us to build spacecraft and go and see, whereas Ian Ridpath (*Messages from the Stars*, Fontana/Collins: London, paperback 95p) is more content to stay put and broadcast our presence and to listen intently for answers or other communications from "out there". Ridpath starts his book by inquiring if life exists elsewhere in space and informing us that "This is the single most important scientific question which we are currently capable of answering, and certainly the most exciting." Well is it? I'm not convinced. It would probably take much more than Little Green Men in Trafalgar Square to shake us from our xenophobic proclivities, and the importance and the excitement of the possibility of there being other peoples than ourselves doesn't seem to be shared by those world scientists intent on the conquest of disease, poverty, famine and war on a

planet whose resources seem to be shrinking as rapidly as its population is exploding.

Ridpath discusses in layman terms the factors that go into the Drake formula (the formula that gives the number of advanced civilisations which exist at the moment and which have the capability for interstellar communication) and the attempts that scientists have already made to find extraterrestrial life, both by experimentation on Mars and by using radio telescopes to listen out. The book considers the humanisation of space and the concepts of space colonies, the message we expect others to send to us, how we would communicate, the consequences of contact, and the possibility that we have already been visited. I found the book a most enjoyable read and a commendable starting point in this engaging subject.

Nicolson's approach is less partisan than Ridpath's. He seems to aim more at the dedicated amateur astronomer than the "man in the street". It is a book which, with exemplary clarity of presentation, brings the reader to and often beyond the frontier between space science and science fiction. Nicolson is discussing star travel, the human race colonising the galaxy. He answers such questions as what spaceships will we use?, "how will they be propelled?", "how long will it take us?", "is it worth it?", and "when will we be ready to go?" Section headings such as "The consequences of contact", "Relativistic starships", "Suspended animation—The practicalities and prospects", "Spinning black holes", "The laser photon sail", "Time dilation", and "The roads to the planets", reveal some of the many feasts that lie in store for the reader. This is an eminently sensible book in a field which is often a happy breeding ground for the over-imaginative. It is a clear, considered scientific exposition of the problems and possibilities of stepping out into the reaches of space. I was impressed. But still the nagging possibility remains that there is no-one out there—no aliens to visit, no reasons to go. What happens if after shouting out our presence, returning to the words of de la Mare, "the silence surged softly backward". I think that realising we are alone would be as disturbing as realising we are not.

Finally we have two books for the telescope owners. I need say very little about Jack Newton's book, *Deep Sky Objects: A Photographic Guide for the Amateur* (Gall Publications: Toronto, hardback £5; paperback £3), other than if you enjoy looking through a telescope, get it immediately. It is a superb practical guide for finding the Messier objects and some of the more interesting of the NGC objects. It contains clear finder charts for all of them and also photographs of each object taken with the author's 12.5-inch Newtonian telescope. Not only are you told where and when to look you are

shown what to expect. All astronomers should say a big thank you to Jack Newton for making their telescope time more productive and more enjoyable.

Using the Telescope, by J. Hedley Robinson (David and Charles: Newton Abbot, UK, £4.95) is a different matter. I will agree with the author that a book in which the amateur observers could find "comprehensive instructions for the proper care and maintenance of their telescopes, together with explicit explanations of correct observational techniques for all celestial bodies that they are likely to be interested in" would be extremely useful. Unfortunately the book under review isn't it. Robinson doesn't go far enough. The budding astrophotographer is told next to nothing about the science of photography and is fobbed off with the 'trial and error method'. The section on telescope mounting is complicated by a

lack of diagrams and by mislabelling the two that are given. When it comes to observational details our appetites are wetted and then we are left up in the air. I'll give you a typical example: "Since then many TLPs have been recorded in a number of craters, both inside and out"—we aren't told which craters. The spectroscopy "is equipped with one or more prisms, set at suitable angles for the light to pass through each in succession," but we are given no hint as to what the angles might be or what prisms to use. I think the amateur observer would do much better by saving a bit more money and buying *Astronomy: A Handbook*, edited by G. R. Roth (Springer: Berlin, 1975).

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Portrait of five heroes

This Wild Abyss: The Story of the Men Who Made Modern Astronomy. By G. E. Christianson. Pp. 461. (Free Press/Macmillan: New York; Collier Macmillan: West Drayton, UK, 1978.) £9.75.

WHAT a pity! as Sir George Sitwell was fond of remarking: such a wasted opportunity. It is a long time since a general book on the history of astronomy was published and it will be some years yet before the new scholarly history (sponsored by the IAU), now in preparation, is before us. Although one's initial expectation that the "makers of modern astronomy" might include such figures as Hubble and Shapley is proved mistaken, a sound survey of the history of astronomy to Newton would be worth having as so much new work has appeared in recent years. There has been the debate about megalithic observatories, many exciting discoveries made about late Islamic astronomy by E. S. Kennedy and others and the outpourings associated with the anniversaries of Copernicus and Kepler, not to mention whole new worlds of scholarship devoted to Galileo and Newton. A new survey of early astronomy could be both more technically competent and more rich in content, as well as more vivid in its discussions of problems, than anything that has appeared yet. Mr Christianson, however, has only had a slight success in releasing himself from a familiar and by now dated pattern.

He makes no claim to be a technical historian of astronomy, which is fair enough; his chief aim has been to write biographies of five great figures: Coper-

nicus, Brahe, Kepler, Galileo and Newton. But he writes, as perhaps he could not but do, about a good deal else including Greek astronomy and medieval civilisation. A general history (even by a non-specialist author) should get the broad historiography and the basic facts right. Mr Christianson tells us that Ptolemy's orbital elements "did not fit comfortably into" the crystalline spheres of Aristotle "and whether or not Ptolemy even believed in such spheres is not known; they receive no mention in his writings". Not so; solid spheres figure prominently in Ptolemy's *Planetary Hypotheses* and are indeed mentioned occasionally in the *Almagest* itself (see O. Pedersen, *Survey of the Almagest*, 1974, 167, 391-7).

Mr Christianson's whole discussion of ancient astronomy is impaired by his failure to grasp the point emphasised seventy years ago by Pierre Duhem that the Greek astronomer was a mathematician, not a philosopher; and correspondingly that it is impossible to appraise the Copernican revolution properly without first seeing the significance of his opposite position that mathematics, far from merely saving the appearances, is an essential key to physical reality. Mr Christianson seems unfortunately to be wholly unaware of the writings of Duhem—widely regarded as providing the basis for the historiography of medieval science as it has flourished during this century—and only ignorance, again, can account for his extraordinary statement that "the study of the Middle Ages" has "until rather recently languished in our institutions of higher education".

Although the author is more familiar with recent studies of Galileo and Newton, his biographies are still marred by serious mistakes, such as the assertion that Galileo discovered the law

of falling bodies by rolling "objects of different weight . . . down an inclined plane . . . keeping time with a specially designed pendulum"; his belief that Descartes proposed to correct chromatic aberration by means of aspherical lenses; and his supposition that Newton plunged deeply into the study of the "occult" (Newton did indeed read deeply in alchemical authors and interpret scriptural prophecy, but no-one has yet made him out to be a warlock).

In general, the ideas of established standard works like T. S. Kuhn's *Copernican Revolution* and Giorgio de Santillana's *Crime of Galileo* are fairly represented, and with Newton the author has made use of Frank Manuel's psycho-analytical portrait, Mrs Dobbs's investigation of Newton's alchemy, the publications of R. S. Westfall, and other recent sources. He ends with an exposition, highly laudatory, of Kuhn's *Structure of Scientific Revolutions* with which, he writes, he agrees that "there is nothing absolute in what is called scientific truth", that is, a statement is true so long as we do not suppose it false.

Mr Christianson has no strong thesis of his own, unlike Arthur Koestler whose *Sleepwalkers* rightly aroused so much interest a few years ago (his ideas too are reflected in this book). Rather, Mr Christianson has attempted to synthesise recent writing in the history of science and particularly the interpretation of the Scientific Revolution. If he is not fully in control of his material this is in part because the history of astronomy has become—largely through the leadership exercised by Otto Neugebauer and his students—a highly technical subject, or rather one should say has been restored to its position as a highly technical subject.

In the personal aspects of his biographies Mr Christianson is on smoother ground, and he shows skill in bringing out the characters and intellectual qualities of his subjects in broad terms. If he sometimes stresses the melodramatic—Tycho's metal nose, Galileo's bastard daughters, Kepler's difficult mother and Newton's neuroses—this is what others have done before, including Koestler. For a philosopher like Galileo to have a mistress, and for a mathematician like Newton to have died a virgin is equally newsworthy. More seriously Mr Christianson presents a reasonably coherent and intelligible, if occasionally flawed portrait of his five heroes and conveys the integrity of the epoch that began with Copernicus and ended with Newton even if (as in any popular account) subtle nuances are absent. **A. Rupert Hall**

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